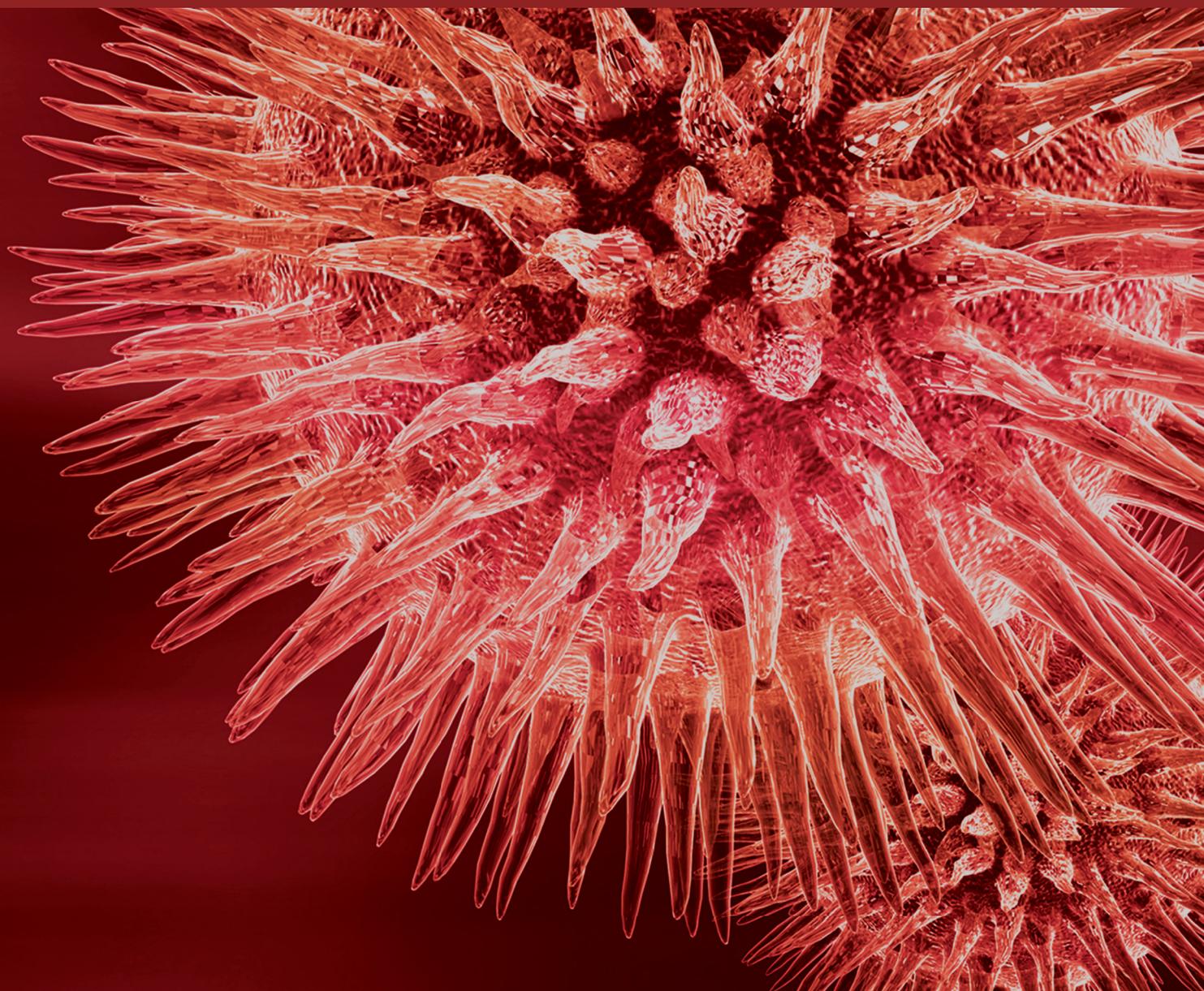


BioMed Research International

Translational Hemorrhagic Stroke: Physiology, Pharmaceutical Drugs, and Management

Lead Guest Editor: Sheng Chen

Guest Editors: John H. Zhang, Mario Zuccarello, Serge Marbacher,
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Editorial

Translational Hemorrhagic Stroke: Physiology, Pharmaceutical Drugs, and Management

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We are pleased to announce the publication of a special issue on major research challenges and achievements in the physiology, pharmaceutical drug therapy, and management of “hemorrhage stroke.” The call for papers on this topic resulted in a significant amount of interest from researchers all over the world. A total of 27 articles were submitted for review. Our editorial team selected 14 basic and clinical search articles for publication and rejected the remaining 13 (an acceptance rate of 52%). Half of the accepted manuscripts are review articles which summarize current literature on diseases such as cervical artery dissection, hydrocephalus after subarachnoid hemorrhage, intracranial atherosclerosis, or hypertensive intracerebral hemorrhage. We are confident that this special issue will advance the understanding and research of the broad range of disorders referred to as “hemorrhagic stroke.”

Acknowledgments

We express our gratitude and recognition to all reviewers who have participated in the review process. Their commitment and willingness to critically evaluate and provide comments on the manuscripts substantially improved each article and have enhanced the overall quality of this special issue.

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Mario Zucarello
Serge Marbacher
Gang Chen
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Review Article

The Roles of Thrombospondins in Hemorrhagic Stroke

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Hemorrhagic stroke is a devastating cerebrovascular disease with significant morbidity and mortality worldwide. Thrombospondins (TSPs), as matricellular proteins, belong to the TSP family which is comprised of five members. All TSPs modulate a variety of cellular functions by binding to various receptors. Recently, TSPs gained attention in the area of hemorrhagic stroke, especially TSP-1. TSP-1 participates in angiogenesis, the inflammatory response, apoptosis, and fibrosis after hemorrhagic stroke through binding to various molecules including but not limited to CD36, CD47, and TGF- β . In this review, we will discuss the roles of TSPs in hemorrhagic stroke and focus primarily on TSP-1.

1. Introduction

Stroke, a major health issue globally, is the second leading cause of death worldwide and the main cause of adult disability [1]. Stroke is divided into two types, according to its etiological mechanisms. Ischemic stroke accounts for the majority of strokes, while hemorrhagic stroke is deadlier and poses a serious public health threat [2]. Intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH), two types of hemorrhagic stroke, are associated with significant morbidity and mortality globally [3–5]. However, the mechanism of brain injury after ICH and SAH is still not fully understood.

The thrombospondin (TSP) family contains five members divided into two subgroups. TSP-1 and TSP-2 compose subgroup A, while TSP-3, TSP-4, and TSP-5 belong to subgroup B. They were shown to regulate cell-cell and cell-matrix interactions by binding to various membrane receptors, other extracellular matrix proteins, and cytokines [6]. In recent years, the research and investigation on TSPs has increased due to their ability to participate in an extensive range of physiological and pathological processes including synaptogenesis, angiogenesis, apoptosis, platelet aggregation, inflammatory response, and wound repair [7, 8].

TSP-1–TSP4 are expressed in the brain [9–11]. They are implicated in synaptogenesis, angiogenesis, and inflammation as well as hydrocephalus after central nervous system (CNS) diseases which includes hemorrhagic stroke [12–23].

2. Methods

TSPs have been getting increased attention in the area of hemorrhagic stroke research. Our literature review indicated that TSPs play diverse roles in hemorrhagic stroke. To better understand their roles in hemorrhagic stroke, the literature search focused mainly on TSP receptors, as there are many. In addition, to elucidate the underlying molecular mechanisms of TSPs in hemorrhagic stroke, we also searched literature regarding TSPs in other diseases, particularly CNS diseases. *PubMed*, *Web of Science*, and *SwetsWise* were the databases utilized in our literature review. After carefully reading the articles, we summarized the roles of TSPs in hemorrhagic stroke and discussed the relevant mechanisms.

2.1. TSP Family. All TSPs are highly homologous at the protein level, especially in their “signature domain,” C-terminal domain, followed by calcium-binding domains, and thirteen EGF-like Type 3 repeats [24]. TSPs are divided into

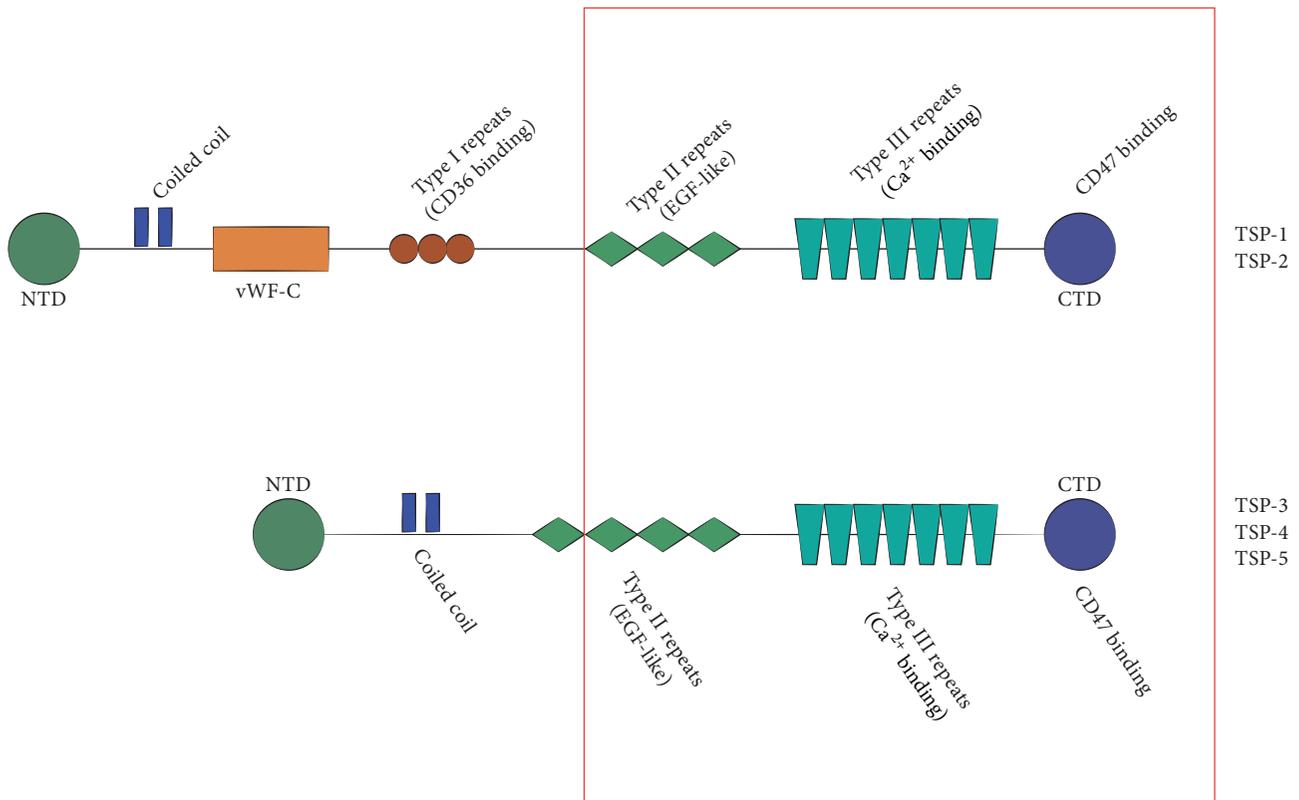


FIGURE 1: Schematic representation of TSPs. All of TSPs share highly homologous CTD, Type 2 repeats, and Type 3 repeats (red region), while TSP-1 and TSP-2 have vWF-C domain and Type 1 repeats. NTD may be characteristic to the family members. TSPs have a complex multidomain architecture that provides an option to bind various ligands. For instance, CTD is involved in CD47 binding, while Type III repeats contain Ca^{2+} binding site. Type I repeats are implicated in interaction with CD36, a receptor for TSP1 and TSP2, and inhibition of MMPs, while vWF-C is responsible for binding members of the TGF- β superfamily. This figure is only a partial listing. CTD: C-terminal domain, EGF-like: epidermal growth factor-like, vWF-C: von Willebrand factor C-type, and NTD: N-terminal domain.

two subgroups due to their disparate N-terminal domains [25] (Figure 1). It is worth mentioning that the transforming growth factor- β (TGF- β) binds to TSP-1 through the vWF-C. Thus, the subgroup B of TSP cannot activate latent TGF- β . The TSP family participates in various physiological and pathological processes through these different functional domains.

TSP-1, one of the members of TSP subgroup A, is a 420–450 kDa trimeric glycoprotein [26, 27]. As a matricellular protein, it was initially isolated from human blood platelets [28, 29]. A variety of cells can produce TSP-1, including endothelial cells (ECs), monocytes/macrophages, smooth-muscle cells, and leukocytes [30–33]. In addition, activated astrocytes can secrete TSP-1 [34, 35]. Certainly, the main sources of TSP-1 are platelets [28]. As a multifunctional protein, TSP-1 has diverse biological roles by interacting with various proteins, cell surface receptors, and proteoglycans of specific domains [36–38] (Table 1).

TSP-2, another member of TSP subgroup A, is similar in structure to TSP-1. It is involved in angiogenesis, synaptogenesis, regulating the cell-matrix interaction, and protecting the integrity of matrix [17, 39]. Some critical differences between TSP-1 and TSP-2 were reported. TSP-1 and TSP-2 were

TABLE 1: Diverse roles of TSP-1.

Binding receptors or molecules	Functions	References
CD36	Antiangiogenic	[13, 45–50]
	Proinflammatory	[15, 51]
	Anti-inflammatory	[17, 45]
	Proapoptotic	[14]
	Profibrogenic	[21, 52]
CD47	Antiangiogenic	[49]
	Anti-inflammatory	[45]
	Proapoptotic	[18, 19]
TNF-R1	Proapoptotic	[20]
TGF- β	Anti-inflammatory	[53]
	Profibrogenic	[54]
MMP-9	Antiangiogenic	[55, 56]
$\alpha 2\delta$ -1, neuroligin 1	Prosynaptogenic	[22, 24]

detected at different time points and had different peaks in a wound healing assay [40]. Moreover, the different expression

of TSP-1 mRNA and TSP-2 mRNA were at disparate time points after ICH [17]. Thus TSP-1 and TSP-2 may exert diverse effects in different stages during ICH.

TSP-3, TSP-4, and TSP-5, belonging to TSP subgroup B, are structurally different from TSP subgroup A (Figure 1). Recently, multiple properties of TSP-4 have been demonstrated, such as having an angiogenic effect and regulating vascular inflammation, extracellular matrix (ECM) remodeling, skin wound healing, and atherosclerotic lesions [41, 42].

2.2. Role of TSPs in Angiogenesis after ICH. As the source of oxygen and glucose for the brain is supplied by the blood, angiogenesis is pivotal for growth and repair of brain [13]. The process of angiogenesis is due to the effect of various proangiogenic and antiangiogenic factors [43]. Antiangiogenic factors are critical for forming new vasculature by inhibiting excessive growth [44]. Wang et al. found an elevated vascular density during retinal vascular development and less susceptibility to hypoxia-mediated disruption of blood vessels in TSP-1 null mice [45]. Furthermore, Bornstein et al. discovered obvious disarrangement of the ECM and denser vascular density during wound repair in TSP-2 null mice [39]. These findings indicate that TSP-1 and TSP-2 are antiangiogenic factors. Subsequently, some researchers found that the expression of TSP-1 and TSP-2 was increased in the process of angiogenesis after ICH [46, 47]. It suggests that TSP-1 and TSP-2 are involved in angiogenesis following ICH.

To explore the role of TSP-1 and TSP-2 in angiogenesis after ICH, Zhou et al. investigated the expression of TSP-1 and TSP-2 after ICH [13]. Abundant TSP-1- and TSP-2-immunoreactive microvessels were observed in the perihematomal region and then extended into the clot after ICH in rat models. Poor immunoreactivity in microvessels was detected in sham-operated group. It suggests that TSP-1 and TSP-2 could inhibit angiogenesis after ICH. In addition, TSP-1 could hinder the vascular endothelial growth factor-(VEGF-) induced angiogenesis via inhibition of NO signaling by interacting with CD47 or CD36. The alteration of TSP-1 might present a negative-feedback mechanism in angiogenesis in hemorrhagic brains [48, 49, 57] (Figure 2).

Paradoxically, there are also evidences that TSP-1 and TSP-2 can promote angiogenesis. In 1994, Nicosia and Tuszynski observed that TSP-1 promoted the formation of microvessels *in vitro* [58]. However, the mechanisms were not characterized. Subsequently, Qian et al. measured the capacity of bovine aortic EC to invade and form microvessel-like tubes in a collagen gel. They showed that TSP-1 can increase EC tube formation at low concentrations but inhibited EC tube formation at higher concentrations [55]. This biphasic effect was related to the stimulation of matrix metalloproteinase-9 (MMP-9) activity by TSP-1. Nevertheless, these results were restricted in that bovine aortic EC and not microvascular EC was used in the experiments. Additional evidence for an angiogenic function of TSP-1 and TSP-2 was found by Yang and colleagues. Yang et al. investigated whether thrombin, a proangiogenic factor, could mediate the expression of TSP-1 and TSP-2 in the brain of ICH rats [17]. The result showed that the expression of TSP-1

and TSP-2 was dramatically decreased after administration of hirudin, a specific thrombin inhibitor, compared with sham-operated animals. On the other hand, intracerebral injection of thrombin markedly increased the expression of TSP-1 and TSP-2. It is rational to speculate that TSP-1 and TSP-2 may promote an angiogenic function by suppressing ECs migration and promoting ECs apoptosis, which leads to the development of new lumen and the maturation of vascular structures [59, 60].

However, the expression of TSP-1 mRNA and TSP-2 mRNA were at diverse time points after ICH [17]. Zhou et al. observed that an obvious increase in TSP-1 mRNA during the early period of ICH might be associated with its inhibitory effect on antiangiogenesis [13]. In contrast, the upregulation of TSP-2 during the later stage following ICH might facilitate the stability of newly formed blood vessels by reducing the level of MMPs and ultimately contribute to the less degradation of the ECM [17, 50, 56]. Taken together, TSP-1 and TSP-2 may execute different effects in angiogenesis after ICH.

In general, these findings indicate that TSPs play a significant role in the process of angiogenesis after ICH. Both TSP-1 and TSP-2 expression were increased after ICH. Previous studies showed that TSP-1 and TSP-2 not only play an antiangiogenic role but also promote angiogenesis. We speculate that the biphasic effect of TSP-1 and TSP-2 may be concentration-related and time-dependent.

2.3. Role of TSP-1 in Inflammation after ICH. After ICH, blood components immediately enter the intracerebral spaces and induce an inflammatory reaction [61]. The inflammatory reaction is involved in depletion of dead cell and other residues and activation of repair signals, a significant defense reaction to brain injury after ICH. A prolonged inflammatory reaction could contribute to detrimental reconstruction [62].

TSP-1 could enhance the release of IL-6 from macrophages by interacting with CD36 in rat myocardial infarction model [51]. NF- κ B can activate interleukin-6 (IL-6), which have crucial role in regulating the immune response by prompting the differentiation of B lymphocyte [63]. Interestingly, the activation of NF- κ B is decreased in macrophages of patients with CD36 deficiency [64]. These results suggest that TSP-1 may induce inflammatory response through NF- κ B pathway. Additional support for a proinflammatory function of TSP-1 has been contributed by Xing and coworkers. Xing et al. showed that exposure of human brain ECs to low concentration of TSP-1 caused a prompt and obvious upregulation in inflammatory adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). This function is done by binding CD47 receptor [15].

Although a lot of experimental studies demonstrate a proinflammatory function of TSP-1, increasing evidence supports an anti-inflammatory function of TSP-1. TSP-1 is a prime activator of TGF- β , which are anti-inflammatory cytokines [65]. Cekanaviciute et al. showed that suppressing astrocytic TGF- β signal contributed to stronger and broader inflammatory response in the peri-infarct cortex after ischemic stroke in mouse model [53]. Furthermore,

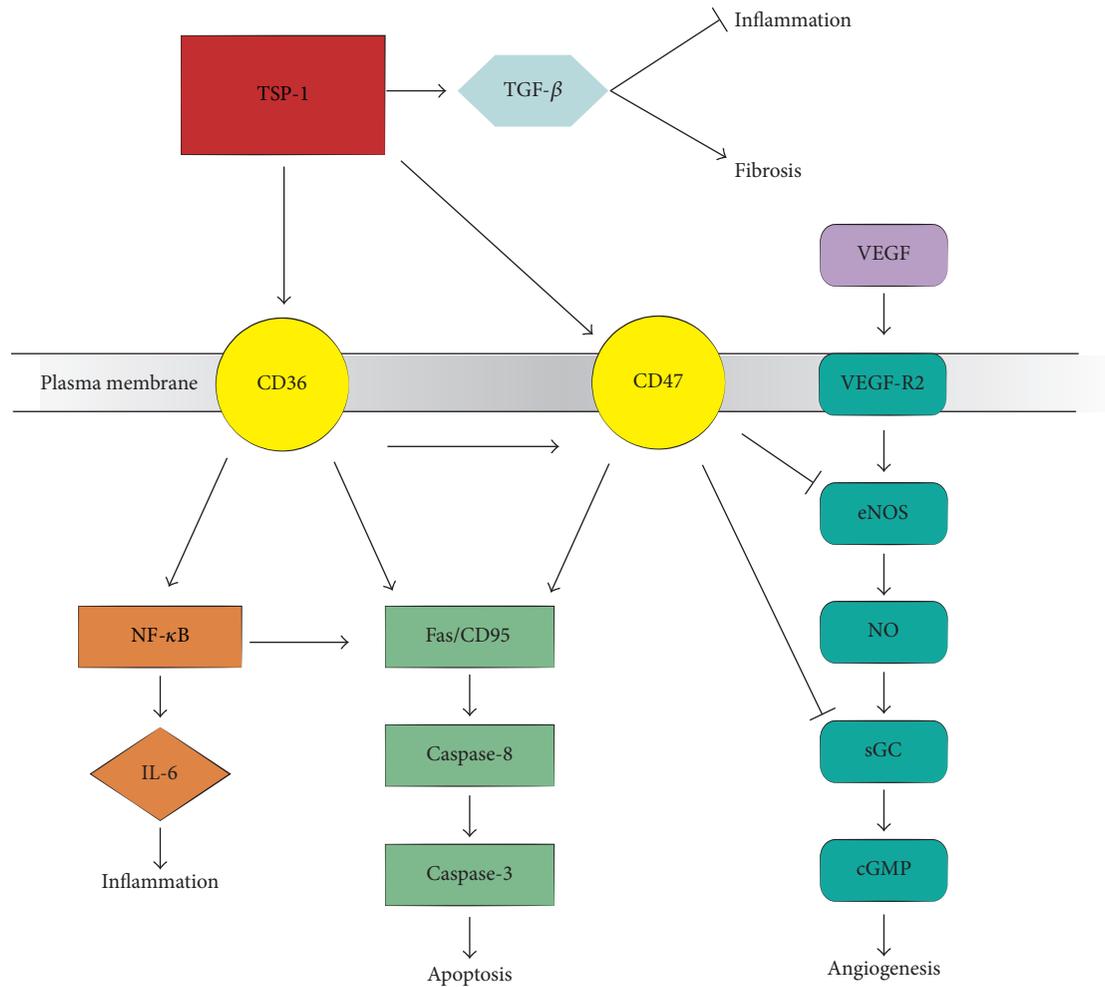


FIGURE 2: The roles of TSP-1 in diverse pathological processes. TSP-1 interacting with CD47 or CD36 suppressed VEGF-induced angiogenesis to inhibit NO signaling pathway. Moreover, TSP-1 binding to CD36 or CD47 induced apoptosis. TSP-1 exerted proinflammatory effect by elevating the levels of IL-6. However, TSP-1 played an anti-inflammatory role by regulating TGF- β and inhibiting NO-mediated vascular cell responses. In addition, TSP-1 mediated fibrosis through a TGF- β signaling pathway.

Yang et al. showed that the expression of TSP-1 was increased at the early phase after ICH, and the upregulation of TSP-1 expression was associated with its inhibitory effect on inflammatory responses after brain injury [17]. The mechanism underlying anti-inflammatory function of TSP-1 may be related to inhibiting NO-mediated vascular cell responses by binding to CD47 or CD36 [45].

In brief, TSP-1 may inhibit the inflammatory response by activating TGF- β or through the NO-mediated vascular cell responses, whereas it may induce inflammatory response through NF- κ B pathway following ICH. It is supposed that the double effects of TSP-1 on inflammatory response are time-dependent. The exact mechanism of TSP-1 on the inflammatory response requires further research.

2.4. Role of TSP-1 in Apoptosis after SAH. Apoptosis is a process of programmed cell death and can also be induced

by multiple pathological stresses. The first time TSP-1 was directly connected to apoptosis was in a cancer study in 1997 [66].

TSP-1 promoted apoptosis in ECs by binding to CD36, which could partially induce cerebrovasospasm after SAH [14]. CD47, another receptor of TSP-1, can promote Fas/CD95-mediated apoptosis in ECs and neutrophils [67, 68]. Another study demonstrated that TSP-1-induced apoptosis in brain microvascular endothelial cells could be regulated by TNF-R1 (tumor necrosis factor receptor 1) [20]. In addition, some evidences showed that the reaction of TSP-1 and CD47 plays a role in neuronal death or survival. Exposure of cultured cerebral cortical neurons to TSP (0.5–10 μ g/mL) for 24 h induced a dose-dependent cell death. Pretreatment with a CD47 blocking antibody for 1h markedly decreased TSP-induced neuronal death through caspase-3-dependent and caspase-independent pathways [18, 19]. It is reasonable to

speculate that TSP-1 plays a role in the process of apoptosis by binding to CD36 or CD47 during SAH.

2.5. Role of TSP-1 in Fibrosis after SAH. SAH not only contributes to vasospasm but also causes subarachnoid fibrosis [69]. Compelling evidences demonstrated that fibrosis of the arachnoid granulations and leptomeninges might contribute to the progress of posthemorrhagic communicating hydrocephalus by decreasing the drainage of cerebrospinal fluid (CSF), hindering the flow of CSF, and reducing CSF absorption [16]. Antifibrinolytic therapy, widely used for reducing the rate of rebleeding in patients with SAH, has not been shown to be associated with the development of hydrocephalus or delayed brain injury after SAH [70].

TSP-1 binds to the small latent complex consisting of the N-terminal prodomain, known as the latency associated peptide (LAP), and the C-terminal portion of the latent complex, known as mature TGF- β [7]. The leucine-serine-lysine-leucine (LKSL) at the amino terminus of LAP that is significant for LAP to interact with the KRFLK sequence of TSP-1 and the modulation of latent TGF- β activation by TSP-1 [54]. In addition, the LKSL peptide could reduce hepatic fibrosis and renal interstitial fibrosis [52, 71, 72]. To determine whether LKSL could protect against subarachnoid fibrosis, Liao et al. investigated the role of LKSL in subarachnoid fibrosis after SAH [21]. Their results revealed that LKSL treatment alleviated subarachnoid fibrosis, delayed the progress of chronic hydrocephalus, and prevented ventriculomegaly formation by suppressing TSP-1-mediated TGF- β signaling pathway. These findings suggest that TSP-1 may serve as a promising target for future therapeutic strategy of subarachnoid fibrosis following SAH.

2.6. Role of TSPs in Synaptogenesis in Other CNS Diseases. Synaptogenesis is important for motor function recovery after various CNS injuries. Increasing evidences suggest that astrocytes are critical in the formation of synapses [73]. TSPs are responsible for the ability of astrocytes to enhance synaptic development *in vitro* [12].

Christopherson et al. observed that both TSP-1 and TSP-2, secreted by astrocytes, could increase CNS synaptogenesis in rat retinal ganglion cells (RGCs) [12]. Moreover, Xu et al. found that TSP-1 promoted synaptogenesis in the early phase of neuronal development in cultured rat hippocampal neurons, but it failed to induce synaptogenesis in mature neurons [22]. This synaptogenic effect of TSP-1 is mediated by neuroligin 1, a membrane protein involved in the formation of CNS synapses [22]. In addition, treatment with TSP-1 increased excitatory synaptogenesis in astrocyte-null hippocampal neuronal cultures [24]. In addition, TSP-4 was expressed in astrocytes, cerebrovascular smooth-muscle cells and ECs and was implicated in adhesion of retinal ganglion cells and axonal outgrowth in the developing retina [74–76].

All TSPs exerted synaptogenic effects by their EGF-like domains directly binding with $\alpha 2\delta$ -1, which were rich in substantial neurons [77, 78]. Overexpression of $\alpha 2\delta$ -1 in neurons increased the number of synapses *in vivo* [77]. However, the role of TSPs in synaptogenesis after hemorrhagic stroke need to be further studied.

2.7. Role of TSP-1 in Evaluation of Severity and Prognosis after Hemorrhagic Stroke

2.7.1. Plasma TSP-1 Concentration. TSP-1 expression was increased after traumatic, ischemic, and hemorrhagic brain injuries in animal cortex [17, 79, 80]. To evaluate the relationship between plasma concentrations of TSP-1 and the severity of hemorrhagic stroke, researchers assessed the levels of TSP-1 in peripheral blood. The results showed that plasma TSP-1 concentration was tightly related to the severity and 6-month clinical outcome following SAH [81]. Additionally, TSP-1 was considered as an independent predictor of 1-week mortality, 6-month mortality, 6-month total survival, and 6-month poor prognosis after ICH [82]. Its predictive value was similar to NIHSS score and hematoma volume under ROC curves [82]. The plasma TSP-1 is thought to be released from circulating blood cells or from the CNS. However, the correlation between plasma TSP-1 levels and platelet count remains unclear [81, 82].

2.7.2. CSF TSP-1 Concentration. Cerebrospinal fluid (CSF) TSP-1 concentration was also altered after hemorrhagic stroke. Chen et al. explored the correlation between CSF TSP-1 concentration and the severity of hemorrhagic stroke [14]. They reported that CSF TSP-1 levels were significantly elevated and reached peak on days 1–3. In addition, others discovered that patients with unfavorable prognosis or vasospasm had higher CSF TSP-1 concentration on days 1–3 and days 5–7 after SAH than those with favorable prognosis or without vasospasm [14]. The increased TSP-1 may be released from leukocytes, platelets in the bloody CSF, or the ECs of the ruptured blood-brain barrier (BBB) and even from the impaired brain tissue [35]. It is believed that TSP-1 may be a potential prognostic biomarker of hemorrhagic stroke.

3. Conclusions and Perspectives

TSPs, as multifunctional proteins, exert diverse functions by binding with various receptors through their distinct domains. TSP-1 participates in angiogenesis, the inflammatory response, apoptosis, and fibrosis following hemorrhagic stroke. Moreover, increased concentration of TSP-1 in both plasma and CSF indicates poor prognosis after hemorrhagic stroke. Future studies are needed to further determine the cellular and molecular mechanisms by which TSP-1 contributes to hemorrhagic stroke. This may lead to the identification of new therapeutic targets.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Clinical Study

Adequate Platelet Function Inhibition Confirmed by Two Inductive Agents Predicts Lower Recurrence of Ischemic Stroke/Transient Ischemic Attack

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Background. The correlation between platelet function and recurrent ischemic stroke or TIA remains uncertain. **Objective.** To investigate two inductive agents to detect platelet functions and assess associations with recurrent ischemic stroke/TIA. **Method.** The study included 738 ischemic stroke/TIA patients. On days 0, 3, and 9 after antiplatelet therapy, platelet function tests were determined by maximum aggregation rate (MAR) using a PL-11 platelet function analyzer and phase matching reagents. Two induction agents were used: arachidonic acid (AA) and adenosine diphosphate (ADP). At 3-month follow-up, recurrence of stroke/TIA was recorded. **Result.** Cut-off values of adequate platelet function inhibition were $MAR_{ADP} < 35\%$ and $MAR_{AA} < 35\%$. Data showed that antiplatelet therapy could reduce the maximum aggregation rate. More importantly, adequate platelet function inhibition of either MAR_{ADP} or MAR_{AA} was not associated with the recurrence of stroke/TIA, but adequate platelet function inhibition of not only MAR_{ADP} but also MAR_{AA} predicts lower recurrence (0/121 (0.00%) versus 18/459 (3.92%), $P = 0.0188$). **Conclusion.** The platelet function tested by PL-11 demonstrated that adequate inhibition of both MAR_{ADP} and MAR_{AA} could predict lower risk of ischemic stroke/TIA recurrence.

1. Introduction

Stroke is the second most common cause of death and is a major cause of disability worldwide [1, 2]. The prognosis of recurrent stroke is worse than first-ever stroke [3, 4]. Antiplatelet therapy is the cornerstone of the secondary prevention of ischemic stroke and transient ischemic attack (TIA) [5]. However, a large number of stroke/TIA patients still experience another cerebral vascular event despite sustained antiplatelet therapy. On average, the annual risk for future ischemic stroke after an initial ischemic stroke/TIA is 3% to 4% [6]. Some researchers interpret such individual difference to antiplatelet drugs as “aspirin/clopidogrel resistance” [7–9]. Therefore, appropriate platelet function measurement to predict aspirin/clopidogrel efficacy is necessary to guide the precise stroke treatment. The current

study is a multicenter study designed to evaluate the platelets' function before and after antiplatelet therapy and analyze the relationship between qualified platelet inhibition and stroke recurrence in the Chinese population.

2. Methods

2.1. Study Design and Patients. The current study is a prospective trial that enrolled 738 patients from 13 stroke centers between October 2014 and December 2015 in China. Inclusion criteria were as follows: (1) age: ≥ 18 years old and ≤ 80 years old; (2) transient ischemic attack (TIA) for the first time, which was defined as a transient episode of neurological dysfunction caused by focal brain or retinal ischemia, without acute infarction symptoms; (3) ischemic stroke for the first

time or recurrent patients with modified Rankin Scale ≤ 2 (ischemic stroke was defined as a sudden neurological deficit, and the infarct site was assessed by magnetic resonance imaging); (4) need for antiplatelet therapy: 100 mg aspirin or 75 mg clopidogrel as monoantiplatelet therapy is initiated within 24 hours after the onset; patients with the following were excluded from the study: (1) intracranial venous thrombosis, (2) cerebral hemorrhage, (3) cerebral embolism, (4) brain tumors, and (5) end-stage severe disease. The main endpoint during the 3-month follow-up was the recurrence of stroke/TIA, bleeding events including cerebral hemorrhage and gastrointestinal bleeding, and other bleeding events.

2.2. Participant Stroke Centers

- (1) The First Affiliated Hospital of Soochow University
- (2) Tianjin Huanhu Hospital
- (3) Peking Union Medical College Hospital (West)
- (4) Weihai Municipal Hospital
- (5) Changhai Hospital
- (6) Shanghai East Hospital
- (7) Lanzhou University Second Hospital
- (8) Zhuhai People's Hospital
- (9) Harrison International Peace Hospital
- (10) The Second Hospital of Tianjin Medical University
- (11) Shijiazhuang Third Hospital
- (12) Affiliated Hospital of North Sichuan Medical College
- (13) Shandong Provincial Hospital

2.3. Key Technology. PL-11 platelet function analyzer (SINNOWA Medical Science & Technology Co., Nanjing, China) is a new point-of-care apparatus for platelet function analysis via an automated impedance technique. Correlations among PL-11 and another three major assays (light transmission aggregometry (LTA), VerifyNow aspirin system, and thromboelastography (TEG)) suggested the ability of PL-11 to assess platelet function.

2.4. Treatment Protocols. All enrolled patients were given aspirin 100 mg/d or clopidogrel 75 mg/d. The medicine would not be changed during the experiment unless patients encountered hemorrhagic or ischemic events or withdrew from the experiment. At the same time, other antiplatelet medicines could not be provided, including Chinese patent medicine containing ingredients like Folium Ginkgo, Salvia Miltiorrhiza, Pseudoginseng, and so on.

2.5. Standard Protocol Approvals, Registration, and Patient Consent. The study protocol was approved by the ethics committee at each study center. Written informed consent was obtained from all participants or their proxies. This trial has been registered in the Chinese Clinical Trials Registry and the registration number is ChiCTR-OCH-14005238.

2.6. Sample Collection and Processing. Antecubital vein blood samples were collected with 3.8% sodium citrate in tubes for monitoring platelet function in all subjects on the day patients were admitted before antiplatelet treatment and 3 days and 9 days after antiplatelet therapy. Blood samples should be stored at room temperature before being tested. The whole procedure required being performed within 2 hours after sampling. Platelet aggregation was detected using PL-11 platelet function analyzer [10] (SINNOWA Medical Science & Technology Co., Nanjing, China).

The whole procedure was automatically done after transferring 500 ml of citrated blood sample into a polycarbonate tube and inserting it into the detecting position. The blood sample in the polycarbonate tube was mixed gently during the whole testing process. Platelet count was detected in duplicate at the start and the mean value of platelet count was set as the baseline. There was a short interval between each test point for system cleaning. 40 μ l of adenosine diphosphate (ADP, 50 μ mol/L) and arachidonic acid (AA, 2 mg/ml) were separately trickled into the blood sample after the second detecting time. The single platelet counting dropped when aggregates formed became too large to be counted as single platelets. PL-11 counted platelets several times until it detected the lowest level. The whole process was finished within 15 min (six detecting times). The system calculated the maximal platelet aggregation ratio according to the following formula:

$$\text{MAR\%} = \frac{(\text{1st platelet count} + \text{2nd platelet count}) / 2 - \text{lowest platelet count}}{(\text{1st platelet count} + \text{2nd platelet count}) / 2} \quad (1)$$

The corresponding maximum aggregation rate of the platelet by each inductive agent was recorded as MAR_{AA} and MAR_{ADP} .

2.7. Statistical Analysis. Baseline characteristics were compared between the ending group (recurrence of ischemic stroke/TIA) and no ending group (nonrecurrence of ischemic stroke/TIA). Continuous variables are presented as mean (standard deviation) and differences were compared using

the analysis of Wilcoxon test. Categorical variables are presented as counts (proportions). Differences were compared using the Fisher test. All tests were 2-sided at a significance level of $P \leq 0.05$ and were performed using SAS software, Version 9.4.

3. Results

3.1. Characteristics of the Patients. From October 2014 through December 2015, we enrolled 738 patients.

TABLE 1: Baseline characteristics of patients by recurrent ischemic stroke/TIA at a 3-month follow-up.

	Ending	No ending	P value
Sex (female), number (%)	14 (66.67)	492 (68.62)	0.8141
Age, years, mean (SD)	63.71 ± 9.01	62.54 ± 10.62	0.3503
Smoking habit, number (%)	7 (33.33)	273 (38.08)	0.8204
SBP, mmHg, mean (SD)	143.76 ± 19.91	147.18 ± 21.89	0.3506
DBP, mmHg, mean (SD)	81.67 ± 9.69	85.35 ± 12.75	0.2093
FPG, mmol/l, mean (SD)	7.21 ± 2.78	7.49 ± 12.97	0.1449
TC, mmol/l, mean (SD)	4.80 ± 1.20	5.99 ± 14.29	0.7442
TG, mmol/l, mean (SD)	1.59 ± 0.54	1.58 ± 1.34	0.1750
BMI, kg/m ² , mean (SD)	23.52 ± 2.03	24.34 ± 2.85	0.1124
Cr, mmol/l, mean (SD)	72.39 ± 29.61	72.94 ± 37.33	0.8156
NIHSS points, mean (SD)*	6.65 ± 4.82	5.25 ± 3.74	0.2866
ABCD points, mean (SD)#	3.25 ± 1.71	3.15 ± 1.34	0.8077

* Compare NIHSS points for patients of ischemic stroke. # Compare ABCD points for patients of transient ischemic attack.

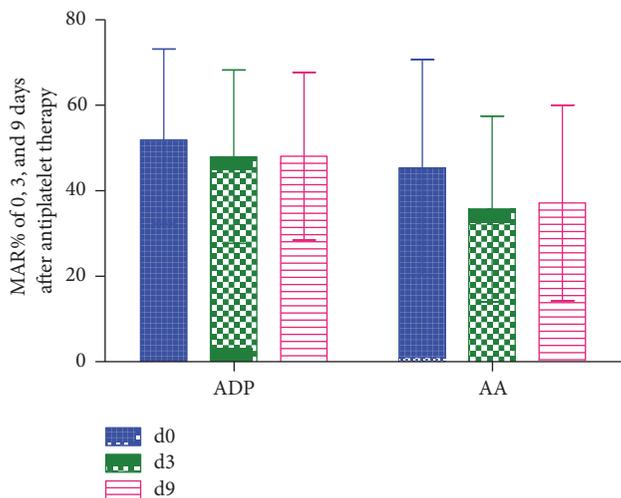


FIGURE 1: MAR% of 0, 3, and 9 days after antiplatelet therapy induced by ADP and AA.

Baseline characteristics of patients by recurrent ischemic stroke/TIA at 3-month follow-up were well matched (Table 1).

3.2. *Antiplatelet Therapy Reduced MAR.* Compared with baseline (Figure 1), MAR_{ADP} was decreased by 9.71% on day 3 (Figure 2, $n = 672$, $47.99\% \pm 20.30\%$ versus $53.15\% \pm 20.72\%$, $P < 0.0001$) and by 9.48% on day 9 (Figure 3, $N = 581$, $48.11\% \pm 19.62\%$ versus $53.15\% \pm 20.72\%$, $P < 0.0001$) after antiplatelet therapy. MAR_{AA} was decreased by 22.37% on day 3 (Figure 2, $n = 664$, $35.77\% \pm 21.72$ versus $46.08\% \pm 25.29\%$, $P < 0.0001$) and by 19.34% on day 9 (Figure 3, $N = 572$, $37.17\% \pm 22.84\%$ versus $46.08\% \pm 25.29\%$, $P < 0.0001$) after antiplatelet therapy.

3.3. *Association between Adequate Platelet Function Inhibition and Recurrence of Stroke/TIA at 3-Month Follow-Up.* The cut-off values of adequate platelet function inhibition were

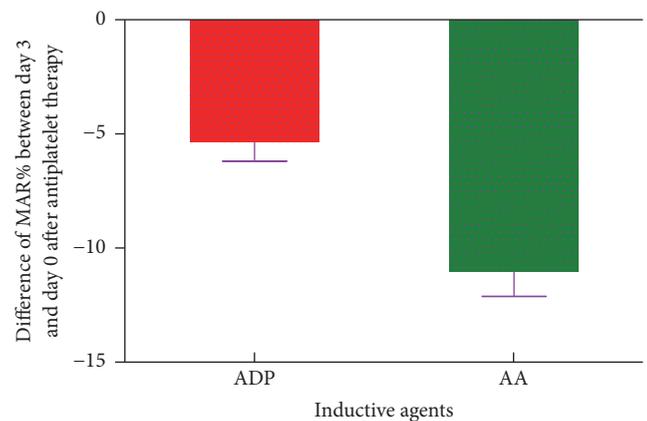


FIGURE 2: Difference of MAR% between day 3 and day 0 after antiplatelet therapy induced by ADP and AA.

MAR_{ADP} < 35% and MAR_{AA} < 35%. Based on these criteria, we divided patients into adequate platelet function inhibition and inadequate inhibition groups. Recurrence of ischemic stroke/TIA at 3-month follow-up was compared between the two groups. When being grouped based on MAR_{ADP}, for patients with MAR_{ADP} < 35%, the recurrence cases were 2 at 3-month follow-up (Table 2, 2/172 (1.16%)), while for patients with MAR_{ADP} ≥ 35%, the recurrence cases were 16 at 3-month follow-up (Table 2, 16/417 (3.84%)). Although the recurrent cases of the adequate platelet function inhibition group were fewer, there was no significant difference between the two groups ($P = 0.0864$). When being grouped based on MAR_{AA}, the recurrence rate was not significantly different either (Table 2, MAR_{AA} < 35% versus MAR_{AA} ≥ 35%, 2.90% (10/345) versus 3.31% (8/242), $P = 0.7782$).

When setting the stricter criteria, take not only MAR_{ADP} < 35% but also MAR_{AA} < 35% as adequate platelet function inhibition. Based on these stricter criteria, we divided patients into adequate platelet function inhibition

TABLE 2: Association of inhibited platelet aggregation with the recurrent ischemic stroke/TIA within a 3-month follow-up.

Inductive agent	Groups (MAR)	Ending (%)	No ending (%)	Numbers	P value
ADP	≥35%	16 (3.84)	401 (96.16)	417	0.0864
	<35%	2 (1.16)	170 (98.84)	172	
AA	≥35%	8 (3.31)	234 (96.69)	242	0.7782
	<35%	10 (2.90)	335 (97.10)	345	
ADP + AA	ADP ≥ 35% or AA ≥ 35%	18 (3.92)	441 (96.08)	459	0.0188
	ADP < 35% and AA < 35%	0 (0.00)	121 (100.00)	121	

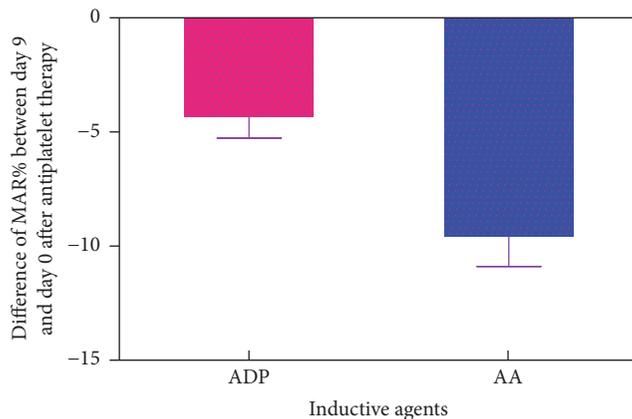


FIGURE 3: Difference of MAR% between day 9 and day 0 after antiplatelet therapy induced by ADP and AA.

and inadequate inhibition groups and compared recurrence of ischemic stroke/TIA between the two groups. At 3-month follow-up, 0.00% (0/121) of the patients experienced recurrence of stroke/TIA in the group of adequate platelet function inhibition, while for the inadequate inhibition group, 18/459 (3.92%) experienced recurrent stroke/TIA. The recurrence rate was significantly different between the two groups (Table 2, $P = 0.0188$).

4. Discussion

The role of antiplatelet therapy in stroke prevention was well documented, especially for secondary prevention [11–13]. But it was also confirmed that there were still a considerable number of patients with ischemic stroke/TIA recurrence even if on treatment of single antiplatelet therapy with aspirin or clopidogrel [5]. This phenomenon may be associated with platelet function. Our study showed that antiplatelet therapy could reduce both MAR_{ADP} and MAR_{AA} in patients of ischemic stroke/TIA. At 3-month follow-up, neither adequate inhibition of MAR_{ADP} nor MAR_{AA} was associated with the recurrence of ischemic stroke/TIA, but adequate inhibition of not only MAR_{ADP} but also MAR_{AA} could predict lower recurrence of ischemic stroke/TIA.

Our study found that MAR_{AA} and MAR_{ADP} significantly decreased in patients of ischemic stroke/TIA after antiplatelet therapy which was consistent with a large number of studies and clinical observations since 2002.

A number of studies had concentrated on the association between platelet function and ischemic events [14]. ARMYDA-Pro [15] and including popular research [16] which was the largest assessment of the predictive value of platelet function tests so far all showed that platelet function was significantly correlated with ischemic vascular outcome. However, several large-scale studies have denied the correlation. TRILOGY-ACS subgroup [17, 18] analysis showed that prasugrel could significantly reduce platelet aggregation but could not reduce cardiovascular mortality, nonfatal myocardial infarction, or stroke within 30 months. Translate-POPs [19] research randomly assigned ACS patients to a strategy of platelet function monitoring, with drug adjustment in patients who had poor responses to antiplatelet therapy, or to a conventional strategy without monitoring and drug adjustment. This study showed no significant improvements in clinical outcomes between the two groups. Similar negative results also had been confirmed by ARCTIC research [20]. Several reasons may account for the different results: (1) platelet function was detected by VerifyNow P2Y12 in some researches, but the method was proven to have low sensitivity [20, 21]; (2) platelets had 6-7 kinds of different receptors and all above researches only measured platelet function by ADP inductive agent, so this may not bring us accurate information [22]. Our study used MAR_{AA} and MAR_{ADP} to evaluate platelet function by PL-11 platelet function analyzer, demonstrating that the single adequate inhibition of either MAR_{ADP} or MAR_{AA} was not associated with the decreased risk of recurrent ischemic stroke/TIA; however, adequate inhibition of both MAR_{ADP} and MAR_{AA} could predict the lower risk of ischemic stroke/TIA recurrence.

The shortcomings of this study are as follows: (1) There is a limitation of MAR_{AA} and MAR_{ADP} by PL-11: the cut-off values of adequate platelet function inhibition by different inductive agents were not confirmed, requiring large cohort studies; (2) the study did not involve adjusting the antiplatelet therapy for ineffective inhibition of platelet function.

Conflicts of Interest

All authors declare no conflicts of interest.

Authors' Contributions

Lulu Zhang and Wei Yue contributed equally to this work.

Acknowledgments

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Research Article

CD36 Gene Polymorphisms Are Associated with Intracerebral Hemorrhage Susceptibility in a Han Chinese Population

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The CD36 gene encodes a membrane glycoprotein (type B scavenger receptor, SR-B2) that plays a crucial role in lipid sensing, innate immunity, atherogenesis, and glycolipid metabolism. In this study, we aimed to investigate the association between CD36 gene polymorphisms and intracerebral hemorrhage (ICH) in a Han Chinese population. We performed genotype and allele analyses for eleven single nucleotide polymorphisms (SNPs) of CD36 in a case-controlled study involving 292 ICH patients and 298 control participants. Eleven SNPs were genotyped by the Improved Multiple Ligase Detection Reaction (iMLDR) method. The results indicated that the SNP rs1194182 values were significantly different between ICH group and control group in a dominant model after adjusting for confounding factors. The subgroup analysis conducted for rs1194182 showed that the allele G frequencies were significantly different between ICH patients and controls in hypertension group via a dominant model. We then analyzed the rs1194182 genotype distributions among different groups of the serum lipid groups, including BMI, TC, TG, HDL, and LDL. However, no significant differences were found in the analysis of other subgroups. Taken together, these findings indicate that rs1194182 polymorphism in the CD36 gene was associated with ICH, and genotype GG could be an independent predictor.

1. Introduction

Intracerebral hemorrhage (ICH) is a crucial classification of stroke and has a high rate of mortality and morbidity [1, 2]. The pathogenesis of ICH is complex and influenced by environmental factors and genetic factors. However, its pathogenesis is not yet clear. Hypertension is the most common contributing factor to primary intracerebral hemorrhage. Factors including smoking, drinking, diabetes, and male gender increase the risk further [3–5]. In addition, many studies have shown an inverse association between the level of serum cholesterol and ICH [6]. More importantly, the role of genetics in the pathogenesis of ICH has received wide attention [7, 8]. Epidemiological studies have demonstrated that a history of a first-degree relative with ICH is an independent risk factor for lobar and nonlobar ICH [4]. It has also been reported that a family history of any stroke was a significant risk factor for patients with ICH who were <70 years compared with those who were >70 years [9]. Taken together,

the observed differences based on family history indicate that genetic factors play an important role in the incidence of ICH.

CD36 is a type B scavenger receptor, now officially designated as SR-B2, and CD36 gene located in the 7q11.2 chromosome with 17 exons and 16 introns and is expressed on the surface of various cells: platelets, microvascular endothelial cells, monocytes/macrophages, dendritic cells, adipocytes, striated muscle cells, and hematopoietic cells [10, 11]. As a result of its expression in multiple cell types, it can be involved in a variety of biological processes, including transport of oxidized LDL (oxLDL) and fatty acids by macrophages and monocytes, and it participates in processes of inflammation, phagocytosis, and endocytosis [12, 13]. In addition, a wide variety of studies have investigated the important roles CD36 plays in many disorders, such as coronary heart disease, hypertension, Alzheimer's Disease, insulin resistance, and metabolic syndrome [14–17]. However, the relationship between CD36 gene polymorphisms and the risk of ICH has not yet been studied. Thus, to clarify the association of CD36

TABLE 1: Demographic and clinical characteristics of ICH patients and controls.

Characteristics	Patients (<i>n</i> = 292)	Controls (<i>n</i> = 298)	<i>P</i> value
Age (years ± SD)	57.12 ± 12.52	58.84 ± 13.61	0.112
Gender, (M/F)	149 (143)	151 (147)	0.931
BMI (kg/m ²)	24.06 ± 3.46	23.44 ± 3.43	0.029
Hypertension, <i>n</i> (%)	227 (77.74)	108 (36.24)	0
Coronary heart disease	27 (265)	21 (238)	0.231
Diabetes mellitus	36 (256)	40 (258)	0.692
Smoking history	69 (223)	59 (239)	0.259
Drinking history	47 (245)	31 (267)	0.041
Family history	12 (280)	4 (294)	0.039
TC (mmol/L)	4.65 ± 1.05	4.10 ± 1.05	0
TG (mmol/L)	2.23 ± 5.41	1.58 ± 1.4	0.049
HDL-c (mmol/L)	1.14 ± 0.33	1.13 ± 0.38	0.712
LDL-c (mmol/L)	3.03 ± 0.76	2.73 ± 0.88	0

with ICH, we conducted this case-control study to find any SNPs of the CD36 gene associated with the risk of ICH.

2. Material and Methods

2.1. Ethics Statement. The study was approved by the local Ethics Committee of Xinqiao Hospital, Third Military Medical University (Chongqing, China), and written consent forms for genetic screening were obtained for all participants from the participant or from their legal representatives.

2.2. Study Population. In this study, a total of 292 patients with ICH were consecutively recruited from Chongqing Xinqiao Hospital from October 2014 to November 2016. All patients were diagnosed with ICH based on results of brain computed tomography (CT) scan and/or magnetic resonance imaging (MRI). The subjects were not eligible if the ICH was caused by trauma, neoplasms, anticoagulant therapy, coagulation disorders, aneurysms, or vascular malformations or if the patient declined to participate in this study. The 298 participants in the control group were randomly selected from the health examination center of Chongqing Xinqiao Hospital during the same period. The inclusion criterion for the controls was the absence of symptoms or medical history of stroke. The baseline characteristics and vascular risk factors were recorded, including age, gender, height, weight, body mass index (BMI), hypertension, coronary heart disease, diabetes mellitus, smoking and drinking habits, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and family history of stroke, which are listed in Table 1. Smokers or drinkers were defined as participants who smoked ≥ 100 cigarettes or drank ≥ 12 times a year. BMI = weight/height² (kg/m²).

2.3. Polymorphism Selection and Genotyping. SNPs were selected based on their functional relevance and minor allele frequency from Beijing (CHB) genotype data in the HapMap database (release #28, August 2010, <https://www.hapmap.org/>). The inclusion criteria were as follows: (1) all eligible

SNP minor allele frequencies > 0.05 in the HapMap database, (2) SNP mutations led to amino acid changes according to the dbSNP, and (3) SNPs were located in one haplotype block and were in complete linkage disequilibrium (LD) (determined with the criterion of $r^2 > 0.8$). Eleven common SNPs spanning the CD36 gene were included. Detailed information of each SNP is shown in Table 2.

Venous blood were collected in EDTA-coated vials after at least a 12-hour overnight fasting period. And the ICH patients' venous blood were collected within 24 h after ICH occurred. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions. Improved Multiple Ligase Detection Reaction (iMLDR) was applied for genotyping [18]. Data analysis was carried out using GeneMapper Software version 4.0. Genotyping was carried out blind to group status.

2.4. Statistical Analysis. Statistical analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). The presence of the Hardy-Weinberg equilibrium was determined using the chi-square test. The categorical variables were tested using the chi-squared (χ^2) test. Categorical variables are expressed as proportions (%) and continuous variables as the mean \pm standard deviation. For continuous variables, all comparisons between ICH group and the control group were made using independent *t*-test and among genotype groups by one-way ANOVA followed by LSD test and multiple comparisons. The genotype and allele distributions in ICH patients and control subjects were determined using the chi-squared (χ^2) test. Multivariate logistic regression was used to analyze the genotype frequency of the subjects specified by different genetic models (additive, dominant and recessive comparison) and to calculate the *P* value, odds ratios (ORs), and 95% confidence intervals (CIs) after adjustment for covariates. Statistically significance was set at $P < 0.05$.

3. Results

A total of 292 ICH patients and 298 control subjects were included in this study. The clinical and laboratory parameters

TABLE 2: Characteristics of CD36 gene polymorphisms investigated in the study.

SNPs	Chromosome	Position	SNP property	Length	Alleles	MAF (CHB_1000 g)
rs1049654	7	80275455	5'UTR	379	A>C	0.325
rs1049673	7	80306350	3'UTR	308	C>G	0.485
rs1194182	7	80231504	5'UTR	256	G>C	0.335
rs12666644	7	80308199	3'UTR	279	G>A	0.087
rs12706949	7	80307224	3'UTR	354	G>T	0.427
rs12706950	7	80307502	3'UTR	244	G>A	0.422
rs13223096	7	80307624	3'UTR	244	C>T	0.427
rs13226433	7	80308171	3'UTR	279	C>A	0.485
rs13246513	7	80306751	3'UTR	250	C>T	0.422
rs1334512	7	80267904	5'UTR	288	G>T	0.272
rs7755	7	80306271	3'UTR	308	G>A	0.485

of all subjects in this study are presented in Table 1. The average age and sex distribution were similar between the ICH group and the control group ($P > 0.05$). As previous studies found, the prevalence of hypertension, drinking history, and family history in the ICH group were significantly higher than in the control group, and there were significant differences between the two groups ($P < 0.05$). BMI values and TC, TG, and LDL-C levels were also slightly raised in ICH patients. There were no significant differences between the two groups in gender, HDL, smoking history, and prevalence of coronary heart disease and diabetes mellitus.

Basic information of the eleven selected SNPs is shown in Table 2. The genotype and allelic frequencies of the SNPs in the ICH group and control group are shown in Table 3. All of the genotypes of eleven SNPs were in agreement with the HWE for the control group ($P > 0.05$), which indicates that the data remain constant in the population (data not shown).

Logistic regression analysis was performed after adjusting for age, gender, body mass index, hypertension, coronary heart disease, diabetes mellitus, smoking and drinking habits, TC, TG, HDL, LDL, and family history of stroke. A significant association between CD36 and ICH was seen in rs1194182, showing that genotype GG was a risk factor for ICH compared with genotype GC-CC (dominant model, OR = 0.674, 95% CI 0.457–0.992, $P = 0.046$). However, allelic frequencies of rs1194182 in the ICH group and control group have no significant difference. In addition, no associations between the other ten SNPs and the risk of ICH were observed in this study.

All the participants were divided into subgroups to examine whether there were associations between CD36 and ICH in gender or hypertension, ICH location, which are listed in Table 4. After logistic regression analysis for rs1194182, the genotype GG distribution (dominant model, OR = 0.578, 95% CI 0.341–0.98, $P = 0.042$) and the allele G frequencies (OR = 1.533, 95% CI 1.095–2.145, $P = 0.012$) were significantly different between ICH patients and controls in the hypertension group. And among nonlobar ICH group, we found a significant difference in the genotype of GG distribution compared to controls (dominant model, OR = 0.645, 95% CI 0.461–0.997, $P = 0.043$). No significant association could be found in the normotensive group, nonlobar ICH group,

the male group, and the female group. CD36 is involved in a variety of roles in lipid metabolism. We then analyzed the rs1194182 genotype distributions among different subgroups, including BMI, TC, TG, HDL, and LDL. However, no significant differences were found in different lipid groups (Table 5).

4. Discussion

In our study, we identified eleven SNPs of CD36 and found that SNP rs1194182 has significant differences in this case-controlled study among a Chinese Han population after adjusting for age, gender, body mass index, hypertension, coronary heart disease, diabetes mellitus, smoking and drinking habits, TC, TG, HDL, LDL, and family history of stroke. Subgroup analysis conducted for the SNP rs1194182 in CD36 showed that GG genotype frequencies were significantly different between ICH patients and controls in hypertension group via a dominant model, especially in the hypertension group.

ICH is an important subtype of stroke and is etiologically diverse. The pathogenesis of ICH involves many factors. As found in our study, CD36 SNP rs1194182 may increase the risk of ICH. However, the mechanism by which CD36 increases the risk of ICH is unknown. It is known that hypertension plays an important role in the pathogenesis of ICH [19], and many studies have found that CD36 is closely related to the development of hypertension [14, 20, 21]. In a microarray analysis study of the differential gene expression between hypertensives and normotensives, 31 genes were upregulated and 18 genes were downregulated, including the CD36 gene with 4.8-fold changes and significant differences between hypertensive and normotensive groups [22]. CD36 deficient individuals were found to have increased blood pressure levels [23]. The +273A/G polymorphism in CD36 was associated with essential hypertension especially in males [14]. Thus, CD36 may contribute to the development of ICH by the following mechanisms. One possibility is through regulating the function of endothelin-1 and altering the properties of vascular smooth muscle, which lead to the development of hypertension and atherosclerosis [24]. Pravenec et al. found that deficiency in renal expression of CD36 could increase blood pressure [21, 25]. In our study, the results showed that

TABLE 3: Comparison of the genotype and allele frequencies of associated CD36 gene variants between ICH patients and controls.

SNPs	Genotype	Frequency		Additive model		Dominant model		Recessive model		Frequency		Multiplicative model		
		ICH patients, n 292(%)	Control patients, n 298(%)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	ICH patients, n (%)	Control patients, n (%)	P	OR (95% CI)	
rs1049654	AA	135 (46.2)	115 (38.6)	0.112	0.798 (0.604-1.054)	0.069	0.698 (0.474-1.028)	0.569	0.849 (0.484-1.49)	A	394 (67.5)	373 (62.6)	0.079	1.24 (0.975-1.576)
	AC	124 (42.5)	143 (48.0)											
	CC	33 (11.3)	40 (13.4)											
rs1049673	CC	60 (20.5)	63 (21.2)	0.847	1.027 (0.783-1.346)	0.894	0.972 (0.638-1.48)	0.632	1.121 (0.703-1.787)	C	265 (45.4)	276 (46.3)	0.748	0.963 (0.766-1.211)
	CG	145 (49.7)	150 (50.3)											
	GG	87 (29.8)	85 (28.5)											
rs1194182	GG	137 (46.9)	118 (39.6)	0.072	0.772 (0.583-1.024)	0.046	0.674 (0.457-0.992)	0.481	0.814 (0.459-1.445)	G	398 (68.2)	378 (63.4)	0.087	0.81 (0.637-1.031)
	GC	124 (42.5)	142 (47.7)											
	CC	31 (10.6)	38 (12.7)											
rs12666644	GG	258 (88.3)	245 (82.2)	0.208	0.723 (0.436-1.198)	0.131	0.664 (0.39-1.13)	0.381	3.211 (0.237-43.56)	G	548 (93.8)	542 (90.9)	0.979	1.517 (0.979-2.35)
	GA	32 (11.0)	52 (17.5)											
	AA	2 (0.7)	1 (0.3)											
rs12706949	GG	83 (28.4)	88 (29.5)	0.787	0.963 (0.734-1.264)	0.509	0.868 (0.57-1.322)	0.786	1.067 (0.667-1.707)	G	315 (53.9)	323 (54.2)	0.93	0.99 (0.787-1.244)
	GT	149 (51.0)	147 (49.3)											
	TT	60 (20.6)	63 (21.2)											
rs12706950	GG	82 (28.1)	88 (29.5)	0.788	0.963 (0.734-1.264)	0.531	0.874 (0.574-1.332)	0.814	1.058 (0.662-1.69)	G	314 (53.8)	322 (54.0)	0.929	0.99 (0.787-1.244)
	GA	150 (51.4)	146 (49.0)											
	AA	60 (20.5)	64 (21.5)											
rs13223096	CC	83 (28.4)	88 (29.5)	0.787	0.963 (0.734-1.264)	0.509	0.868 (0.57-1.332)	0.786	1.067 (0.667-1.707)	C	315 (53.9)	323 (54.2)	0.93	0.99 (0.787-1.244)
	CT	149 (51.0)	147 (49.3)											
	TT	60 (20.6)	63 (21.2)											
rs13226433	CC	59 (20.2)	62 (20.8)	0.833	1.03 (0.785-1.351)	0.869	0.965 (0.634-1.469)	0.584	1.14 (0.713-1.825)	C	264 (45.2)	275 (46.1)	0.747	0.963 (0.766-1.211)
	CA	146 (50.0)	151 (50.7)											
	AA	87 (29.8)	85 (28.5)											
rs13246513	CC	83 (28.4)	89 (29.9)	0.798	0.965 (0.735-1.267)	0.568	0.885 (0.528-1.346)	0.482	1.049 (0.655-1.68)	C	316 (54.1)	324 (54.4)	0.931	0.99 (0.787-1.245)
	CT	150 (51.4)	146 (49.0)											
	TT	59 (20.2)	63 (21.1)											
rs13344512	GG	172 (58.9)	188 (63.1)	0.11	1.302 (0.942-1.8)	0.246	1.259 (0.583-1.859)	0.09	2.203 (0.884-5.487)	G	441 (75.5)	477 (80.0)	0.062	0.769 (0.584-1.013)
	GT	97 (33.2)	101 (33.9)											
	TT	23 (7.9)	9 (3.0)											
rs7755	GG	60 (20.6)	63 (21.2)	0.922	1.014 (0.773-1.328)	0.78	0.942 (0.619-1.433)	0.632	1.121 (0.703-1.787)	G	264 (45.2)	276 (46.3)	0.704	0.957 (0.761-1.203)
	GA	144 (49.3)	150 (50.3)											
	AA	88 (30.1)	85 (28.5)											

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism. Additive model: heterozygotes and minor allele homozygotes were weighed 2 and 1, respectively; to major allele homozygotes. Dominant model: major allele homozygotes versus heterozygotes plus minor allele homozygotes. Recessive model: major allele homozygotes plus heterozygotes versus minor allele homozygotes.

TABLE 4: The genotype distributions and allele frequencies of the CD36 gene rs1194182 polymorphisms in the hypertension and normotensive groups.

Subgroup	rs1194182			Additive model			Dominant model			Recessive model			Allele			Multiplicative model	
	GG	GC	CC	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	G	C	P	OR (95% CI)		
Male (ICH)(149)	69	64	16	0.613	0.905 (0.161-1.331)	0.601	0.866 (0.505-1.485)	0.79	0.9	0.242	1.223 (0.873-1.713)	202	96	0.242	1.223 (0.873-1.713)		
Male (control)(151)	62	67	22									191	111				
Female (ICH)(143)	68	60	15	0.601	0.682 (0.33-1.409)	0.201	0.498 (0.171-1.45)	0.791	0.83 (0.209-3.293)	0.21	1.246 (0.883-1.759)	196	90	0.21	1.246 (0.883-1.759)		
Female (control)(147)	56	75	16									187	107				
Hypertension (ICH)(227)	105	100	22	0.053	0.696 (0.482-1.005)	0.042	0.578 (0.341-0.98)	0.333	0.7	0.012	1.533 (1.095-2.145)	310	144	0.012	1.533 (1.095-2.145)		
Hypertension (control)(107)	36	53	18									125	89				
Normotensive (ICH)(65)	32	24	9	0.982	1.005 (0.64-1.58)	0.539	0.828 (0.453-1.513)	0.307	1.602 (0.648-3.96)	0.76	1.068 (0.699-1.633)	88	42	0.76	1.068 (0.699-1.633)		
Normotensive (control)(191)	82	89	20									253	129				
Lobar (ICH)(40)	18	19	3	0.973	1.018 (0.761-1.631)	0.604	0.947 (0.622-1.846)	0.423	1.098 (0.874-2.96)	0.351	1.269 (0.769-2.095)	55	25	0.351	1.269 (0.769-2.095)		
Lobar (control)(298)	118	142	38									378	218				
Nonlobar (ICH)(252)	119	105	28	0.063	0.778 (0.311-1.592)	0.043	0.645 (0.461-0.997)	0.512	0.797 (0.384-1.524)	0.107	1.229 (0.956-1.579)	343	161	0.107	1.229 (0.956-1.579)		
Nonlobar (control)(298)	118	142	38									378	218				

TABLE 5: The BMI and serum lipid data of ICH patients and control subjects stratified by CD36 genotypes.

Parameter	Genotype			P value (multiple comparison)
	GG	GC	CC	
BMI (ICH)	24.043 ± 3.423	24.311 ± 3.563	23.651 ± 4.042	0.59
BMI (control)	23.367 ± 3.053	23.479 ± 3.602	23.533 ± 3.916	0.952
TC (ICH)	4.7045 ± 1.112	4.649 ± 1.041	4.394 ± 0.748	0.332
TC (control)	4.117 ± 1.154	4.134 ± 0.927	3.908 ± 1.173	0.489
TG (ICH)	1.926 ± 1.671	1.927 ± 1.223	1.886 ± 1.109	0.989
TG (control)	1.64 ± 1.52	1.609 ± 1.413	1.292 ± 0.88	0.391
HDL (ICH)	1.174 ± 0.338	1.111 ± 0.316	1.149 ± 0.303	0.295
HDL (control)	1.113 ± 0.295	1.152 ± 0.451	1.131 ± 0.317	0.711
LDL (ICH)	3.076 ± 0.768	3.037 ± 0.794	2.841 ± 0.61	0.303
LDL (control)	2.739 ± 0.843	2.728 ± 0.919	2.627 ± 0.841	0.717

rs1194182 polymorphism in the CD36 was significantly associated with ICH in the hypertension group, which indicated that significant correlation existed between rs1194182 polymorphism and hypertension on ICH. Moreover, previous study found that ICH occurring in different location may have different pathophysiology, among all cases of lobar ICH, which mainly caused by an apolipoprotein E4 or E2 allele. But about half of all cases of nonlobar ICH are attributable to hypertension [4]. Thus, we assume that CD36 gives rise to ICH through blood pressure pathways. In addition, SNP rs1194182 in the CD36 gene could be a molecular marker for ICH particularly in hypertensive patients.

CD36 is involved in a variety of lipid metabolism pathways. CD36 has the ability to facilitate the uptake of long-chain fatty acids in muscle and adipose tissues, which contributes to the regulation of lipid metabolism and insulin resistance [26, 27]. CD36 is also expressed on macrophages as a receptor for oxidized low-density lipoproteins and plays an important role in the development of atherosclerosis [28]. Previous Japanese studies have reported an association between rare CD36 variants and high blood levels of free fatty acid and triglycerides [29, 30]. CD36 sequence variants were also associated with HDL-C levels [31]. A meta-analysis found that the CD36 gene was significantly linked to triglycerides and triglycerides/HDL-C ratio but not linked to LDL or total cholesterol [32]. In addition, many studies have demonstrated that the polymorphism of CD36 influences the serum lipid levels in the patients of atherosclerosis, coronary heart disease, and metabolic syndrome [33–35]. Importantly, as the Rotterdam Study found, low serum triglycerides levels were also associated with an increased risk of ICH [36]. To evaluate the influence of those factors on the genotype of CD36 in this study, we conduct subgroups analysis according to the BMI, TC, TG, HDL, and LDL. However, for rs1194182, there was no significant difference among lipid subgroups found in the analysis. These findings may indicate that the CD36 increased risk of ICH is not through dyslipidemia pathways.

To our knowledge, this study was the first to report an association between SNPs of CD36 and ICH in a Chinese

Han population. This association may be stronger for those individuals labeled as ICH. The SNP rs1194182 in CD36 can be recognized as molecular markers of ICH even though the mechanism is not clear. However, the main limitations of this study are the small sample size and the mechanisms of the CD36 gene in ICH are still unknown. These results should be validated in a large population and in different ethnicities. In our future studies, we will work on the mechanisms of the CD36 gene in ICH with a larger sample size in more diverse areas. As the association between SNPs of CD36 and ICH has been confirmed in a larger sample size and more diverse areas, we would estimate individual risk of ICH just according the venous blood sample.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qiu-Wen Gong, Mao-Fan Liao, and Liang Liu contributed equally to this work.

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Review Article

Current Status and Future Perspective of Stenting for Symptomatic Intracranial Atherosclerotic Disease: A Meta-Analysis

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The aim of this study was to evaluate the safety and effectiveness of percutaneous transluminal angioplasty and stenting (PTAS) for intracranial atherosclerotic disease (ICAD) by conducting a meta-analysis. Two independent observers searched PubMed, EMBASE, and Cochrane Library for relevant studies up to 31 December 2016. A meta-analysis was conducted using Review Manager 5.3. Three studies involving 581 cases were included. The meta-analysis indicated that any stroke (RR = 3.13; 95% CI: 1.80–5.42), ischemic stroke (RR = 2.15; 95% CI: 1.19–3.89), and intracranial hemorrhage (RR = 14.71; 95% CI: 1.96–110.48) within 30 days in medical therapy alone were lower compared with PTAS plus medical therapy, but there were no significant differences in any stroke and ischemic stroke beyond 30 days between the two groups. There were also no significant differences in any death and myocardial infarction between the two groups. This meta-analysis demonstrated that, compared with medical therapy alone, PTAS for ICAD had a high risk of complication, but most complications in PTAS group occurred within 30 days after the operation, and beyond 30 days the PTAS was not inferior compared with medical therapy alone. Further studies are needed to reduce the periprocedural complications and reappraise the PTAS.

1. Introduction

Intracranial atherosclerotic disease (ICAD) is a common cause of stroke and associated with a high risk of recurrent stroke [1, 2]. Its incidence and prevalence vary by ethnicity. ICAD is more common in Asians, Hispanics, and those of African descent, compared to Caucasians. It causes approximately 10% of all strokes in the USA [3, 4]. In Asian studies, ICAD accounts for 33–50% of all strokes in China, 47% in Thailand, 48% in Singapore, and 10–25% in Korea [5]. In National Institute of Health-sponsored, multicenter Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) trial [6], 14% and 23% of the patients with a transient ischemic attack (TIA) or stroke attributable to a high-grade (50–99%) intracranial stenosis had a further ipsilateral

ischemic stroke over the next year despite medical therapy. Consequently, alternative therapies are urgently needed for these patients.

Over the past decade, intracranial percutaneous transluminal angioplasty and stenting (PTAS), including the use of balloon-mounted stent or self-expanding stent, has increasingly been used in clinical practice all around the world [7–9]. The first randomized trial, stenting versus aggressive medical therapy for intracranial arterial stenosis (SAMMPRIS) trial, was reported in 2011 [10]. The rate of 30-day stroke or death in PTAS group was 14.7%, which was much higher than expected and implied that aggressive medical management was superior to PTAS. Criticisms regarding the design in SAMMPRIS have been raised including the inexperience of the operators and poor patient selection. Lessons learned

from SAMMPRIS changed the design on patient selection, stenting techniques, and periprocedural management [11]. After that several well designed clinical trials were reported, and several trials showed a lower rate of 30-day stroke or death. Against the background of an increasing amount of data on this endovascular therapy field, we systematically searched the relevant studies, which compared the immediate and long term outcomes between PTAS plus medical therapy and medical therapy alone for ICAD.

2. Methods

2.1. Inclusion Criteria and Exclusion Criteria. Studies were considered for inclusion if they met the following criteria: (1) all published randomized, controlled trials (RCTs) were comparing PTAS plus medical therapy and medical therapy alone and more than 3 patients enrolled in each group; (2) all patients had been treated for an atherosclerotic intracranial stenosis greater than 50% which located in intracranial segment of internal carotid artery, middle cerebral artery, and vertebral or basilar artery; and (3) periprocedural complications were reported. Studies were considered for exclusion if they met the following criteria: (1) follow-up time was less than 1 year and (2) complication rate could not be extracted.

2.2. Search Strategy and Data Extraction. Two independent observers searched PubMed, EMBASE, and Cochrane Library for the relevant studies published in English up to 31 December 2016. The key words included “intracranial arteriosclerosis”, “cerebral arteriosclerosis”, “stenosis”, “stent”, and “randomized controlled trial”. Two reviewers independently reviewed the citations, abstracts, and full-text articles and determined the eligibility of all the studies identified in the initial search. When the entire process was completed, the two cross-checked with each other. In cases of disagreements, a third reviewer was consulted. We systematically reviewed any stroke, ischemic stroke, intracranial hemorrhage, any death, myocardial infarction, and so on during follow-up period reported in all the trials. For RCTs, the following details were extracted: participants, follow-up time, eligibility criteria, stenosis rate, stenosis location, and primary end points. Articles that met all inclusion criteria but specific data extraction was not possible were marked as “NG” (not given). After systemic review, data of any stroke, ischemic stroke, intracranial hemorrhage, any death, and myocardial infarction within 30 days and during the follow-up were used in meta-analysis.

2.3. Quality Assessment. Assessment of the quality of the included studies was performed using the methodology recommended by Cochrane Handbook for Systematic Reviews of Interventions [12]. This method comprised assessments of the risk of potential bias in seven domains: random sequence generation (low risk, high risk, or unclear risk), allocation concealment (low risk, high risk, or unclear risk), blinding of outcome assessment (low risk, high risk, or unclear risk), blinding of participants and personnel (low risk, high risk, or unclear risk), incomplete outcome data (low risk, high risk, or unclear risk), selective reporting (low risk, high risk, or

unclear risk), and other biases (low risk, high risk, or unclear risk), such as the baseline, source of funding, and academic biases. Two reviewers will independently assess the quality of the included trials. Discrepancies will be resolved by mutual consensus with a third author.

3. Statistical Analysis

Statistical analysis was performed using Review Manager Version 5.3 software (Cochrane Collaboration, Oxford, UK). We conducted separate meta-analysis according to different groups. The heterogeneity of the qualitative analysis was assessed by Chi-square test, and the significant level was set to $P = 0.1$. We used I^2 to conduct quantitative analysis of heterogeneity. The significant level was set to 50%. If $P > 0.1$ and $I^2 < 50\%$, the different RCTs can be regarded as homogeneous. If $P < 0.1$ and $I^2 \geq 50\%$, the different RCTs can be regarded as heterogeneity. All pooled effect estimates were assessed using random effects model. We used weighted mean deviation (WMD) and 95% confidence interval (CI) to represent the continuous data, and the dichotomous data can be described by risk ratio (RR) and 95% CI. Our meta-analysis has been registered (URL: <https://www.crd.york.ac.uk/PROSPERO/>; unique identifier: CRD42015024370).

4. Results

The 151 potentially relevant trials were identified from the databases in the initial search, and then 36 duplicate trials were excluded. The search identified 115 citations. Finally, only 3 studies involving 581 cases met the inclusion criteria (Figure 1). All the 3 studies—the Stenting and Aggressive Medical Management for Preventing Recurrent stroke in Intracranial Stenosis (SAMMPRIS) trial was reported in 2011 [10] and 2014 [13], the Vitesse™ Intracranial Stent Study for Ischemic Stroke Therapy (VISSIT) trial was reported in 2015 [14], and Vertebral Artery Stenting trial (VAST) was reported in 2015 [15]—described participants, follow-up time, eligibility criteria, stenosis rate, stenosis location, and primary end points (Table 1). For SAMMPRIS, with regard to data within 30 days we used the data published in 2011, because this article was written when the last patient enrolled completed the 30-day evaluation, and, with regard to data in 1 year or longer, we used the data published in 2014 because this was the final result of SAMMPRIS trial.

4.1. Quality Assessment of the Included RCTs. All 3 RCTs mentioned “random” and described the method of generating a random sequence. Because only one of the treatment groups underwent stenting, the trial could not be double masked. All of the studies described the case where subjects quit or were lost to follow-up. The number of subjects that quit or were lost to follow-up of each study were less than 20% of the total number. Therefore, we considered the data integrity was good. The detailed assessments are shown in Figure 2.

4.2. Any Stroke within 30 Days, beyond 30 Days, between 30 Days and 1 Year, within 1 Year, and during the Follow-Up. All of the 3 studies reported any stroke (including ischemic stroke

TABLE 1: Characteristics of the RCTs, comparing PTAS plus medical therapy with medical therapy alone.

	Participants	Follow-up	Eligibility criteria	Stenosis rate	Stenosis location	Primary end point
SAMMPRIS	451	32.4 months	TIA or nondisabling stroke within 30 days	70%–99%	Major intracranial arteries	Any stroke or death, myocardial infarction, and any major hemorrhage
VISSIT	111	1 year	Hard TIA or stroke within the past 30 days	70%–99%	Intracranial internal carotid, middle cerebral, intracranial vertebral, or basilar arteries	Any stroke or death, hard TIA, NIHSS, and mRS scores
VAST	19 [†]	3 years	Vertebrobasilar TIA or minor ischemic stroke in the previous 6 months	≥50%	Intracranial vertebral arteries	Vascular death, myocardial infarction, or any stroke

[†]The VAST included 115 patients but only 19 of them were located in intracranial vertebral artery; SAMMPRIS: Stenting and Aggressive Medical Management for Preventing Recurrent stroke in Intracranial Stenosis; VISSIT: Vitesse Intracranial Stent Study for Ischemic Stroke Therapy. VAST: Vertebral Artery Stenting Trial; TIA: transient ischemic attack; NIHSS: National Institute of Health Stroke Severity Scale; mRS: modified Rankin Scale.

and hemorrhage stroke) within 30 days, and the SAMMPRIS and VISSIT trials also reported any stroke within 1 year and during the follow-up. The median duration of follow-up in SAMMPRIS trial was 32.4 months (IQR 24.2–40.5; range: 0–52.6 months); and the median follow-up time in VISSIT trial was 10.5 months (range: 0–51 months). Comparing PTAS plus medical therapy with medical therapy alone, there was no heterogeneity from any stroke within 30 days ($P = 0.77$; $I^2 = 0\%$), any stroke beyond 30 days ($P = 0.26$; $I^2 = 21\%$), and any stroke between 30 days and 1 year ($P = 0.22$; $I^2 = 34\%$). The pooled results showed significant difference in any stroke within 30 days (RR = 3.13; 95% CI: 1.80–5.42; Figure 3) but had no significant differences in any stroke beyond 30 days (RR = 1.04; 95% CI: 0.51–2.11; Figure 4) and between 30 days and 1 year (RR = 1.03; 95% CI: 0.41–2.56; Figure 5). There was heterogeneity from any stroke within 1 year ($P = 0.07$; $I^2 = 69\%$) and any stroke during the follow-up ($P = 0.06$; $I^2 = 73\%$) between two groups. The pooled results showed significant difference in any stroke within 1 year (RR = 2.12; 95% CI: 0.89–5.03; Figure 6) and any stroke during the follow-up (RR = 2.07; 95% CI: 0.83–5.16; Figure 7).

4.3. Ischemic Stroke within 30 Days, beyond 30 Days, and during the Follow-Up. The SAMMPRIS and VISSIT trials reported the ischemic stroke as serious adverse events within 30 days and during the follow-up, including ischemic stroke in the territory of qualifying symptomatic artery and ischemic stroke not in the territory of qualifying symptomatic artery and cerebral infarction. Comparing PTAS plus medical therapy with medical therapy alone, there was no heterogeneity from the ischemic stroke within 30 days ($P = 0.53$; $I^2 = 0\%$) and beyond 30 days ($P = 0.22$; $I^2 = 34\%$). The pooled results showed significant difference in ischemic stroke within 30 days (RR = 2.15; 95% CI: 1.19–3.89; Figure 8) but no significant difference in ischemic stroke beyond 30 days (RR = 1.02 95% CI: 0.42–2.45; Figure 9). There was heterogeneity from the ischemic stroke during the follow-up ($P = 0.09$; $I^2 = 65\%$) between two groups. The pooled

results showed no significant difference (RR = 1.59; 95% CI: 0.69–3.66; Figure 10).

4.4. Intracranial Hemorrhage within 30 Days and during the Follow-Up. The SAMMPRIS and VISSIT trials reported the intracranial hemorrhage as serious adverse events during the follow-up, including intracranial hematoma, symptomatic intracranial hemorrhage, and asymptomatic intracranial hemorrhage. Comparing PTAS plus medical therapy with medical therapy alone, there was no heterogeneity from the intracranial hemorrhage within 30 days ($P = 0.71$; $I^2 = 0\%$) and during the follow-up ($P = 0.80$; $I^2 = 0\%$). The pooled results showed significant differences in intracranial hemorrhage within 30 days (RR = 14.71; 95% CI: 1.96–110.48; Figure 11) and during the follow-up (RR = 7.20; 95% CI: 1.94–26.77; Figure 12).

4.5. Any Death within 30 Days, beyond 30 Days, between 30 Days and 1 Year, within 1 Year, and during the Follow-Up. The SAMMPRIS and VISSIT trials reported any death within 30 days, beyond 30 days, between 30 days and within 1 year, within 1 year, and during the follow-up. Comparing PTAS plus medical therapy with medical therapy alone, there was no heterogeneity from any death within 30 days ($P = 0.51$; $I^2 = 0\%$), beyond 30 days ($P = 0.55$; $I^2 = 0\%$), between 30 days and 1 year ($P = 0.61$; $I^2 = 0\%$), within 1 year ($P = 0.54$; $I^2 = 0\%$), and during the follow-up ($P = 0.52$; $I^2 = 0\%$). The pooled results showed no significant differences in any death within 30 days (RR = 1.14; 95% CI: 0.43–3.00; Figure 13), beyond 30 days (RR = 0.88; 95% CI: 0.32–2.41; Figure 14), between 30 days and 1 year (RR = 0.73; 95% CI: 0.16–3.31; Figure 15), within 1 year (RR = 1.16; 95% CI: 0.52–2.57; Figure 16), and during the follow-up (RR = 1.12; 95% CI: 0.57–2.21; Figure 17).

4.6. Myocardial Infarction during Follow-Up. The SAMMPRIS and VISSIT trials reported the myocardial infarction during follow-up. Comparing PTAS plus medical therapy

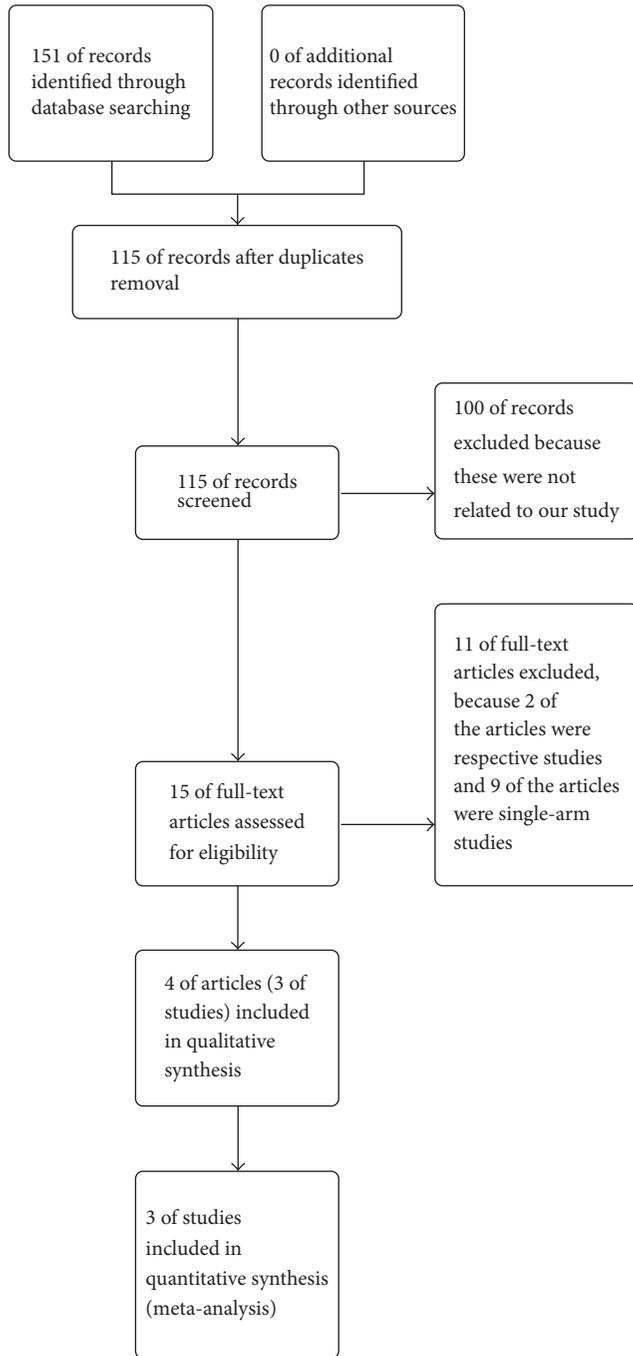


FIGURE 1: Flow diagram of the controlled trials reviewed for this meta-analysis.

with medical therapy alone, there was no heterogeneity from the myocardial infarction during follow-up ($P = 0.35$; $I^2 = 0\%$). The pooled results showed no significant difference in myocardial infarction during follow-up (RR = 0.66; 95% CI: 0.24–1.84; Figure 18).

5. Discussion

This meta-analysis indicated that any stroke, ischemic stroke, and intracranial hemorrhage within 30 days in medical

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other biases
Chimowitz et al., 2011	+	?	-	+	+	+	+
Compter et al., 2015	+	?	-	+	+	+	+
Derdeyn et al., 2014	+	?	-	+	+	+	+
Zaidat et al., 2015	+	?	-	+	+	+	+

FIGURE 2: Quality assessment for included RCTs.

therapy alone were lower, compared with PTAS plus medical therapy. But there were no significant differences in any stroke and ischemic stroke beyond 30 days between two groups. This indicated that stroke in PTAS plus medical therapy occurred in early period after operation. The SAMMPRIS trial was the first randomized trial to compare PTAS plus medical therapy with medical therapy alone. This trial enrolled 451 patients who had a TIA or nondisabling stroke within 30 days attributed to angiographically verified stenosis of 70 to 99% of the diameter of a major intracranial artery at 50 sites in the United States, and PTAS was performed under general anesthesia with the Gateway PTA Balloon Catheter and Wingspan Stent System [10, 13]. The high 30-day rate of stroke or death in PTAS group was the main reason for bad outcomes in the PTAS group, and 75% (25/33) of the events occurred within 24 hours of stenting [16] implying the flaws in study design (such as the patient selection). Dramatically, beyond 30 days the rate of stroke or death was not significantly different between the two groups [13], similar to the results of our meta-analysis, which meant that the PTAS was safe for long time follow-up. Similarly since we could not obtain the results of intracranial hemorrhage beyond 30 days, we still concluded the intracranial hemorrhage occurred in early period after operation according to the results of intracranial hemorrhage within 30 days and during follow-up.

Patients in the VISSIT trial had symptomatic intracranial stenosis (70%–99%) involving internal carotid, middle cerebral, intracranial vertebral, or basilar arteries and had a transient ischemic attack (TIA) or nondisabling stroke attributable to the territory of the target lesion within the past 30 days, and this trial was terminated due to the low

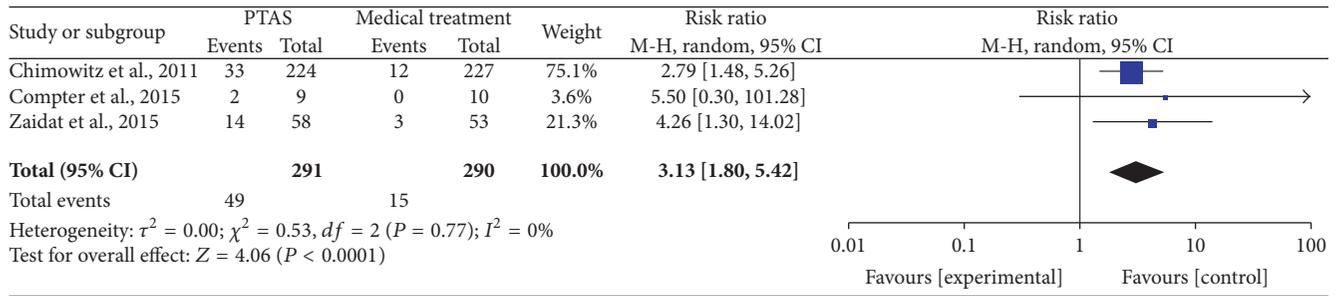


FIGURE 3: Forest plot of any stroke within 30 days for PTAS plus medical therapy versus medical therapy alone.

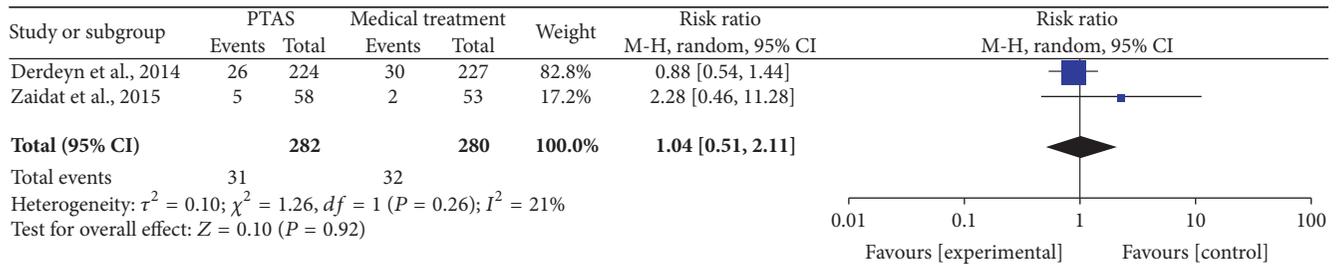


FIGURE 4: Forest plot of any stroke beyond 30 days for PTAS plus medical therapy versus medical therapy alone.

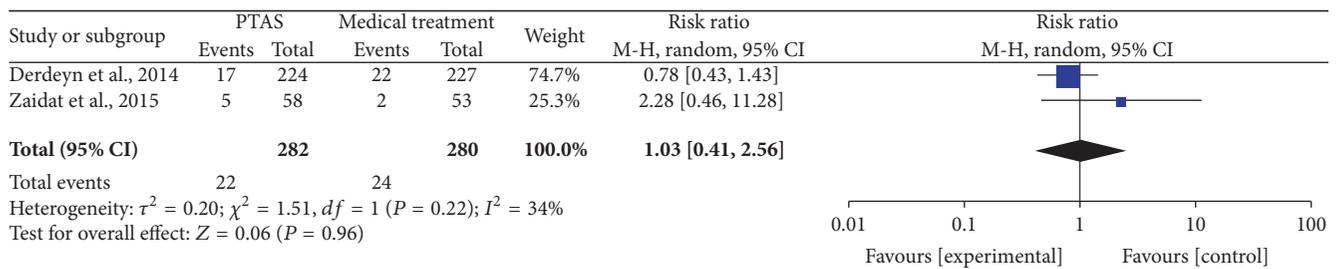


FIGURE 5: Forest plot of any stroke between 30 days and 1 year for PTAS plus medical therapy versus medical therapy alone.

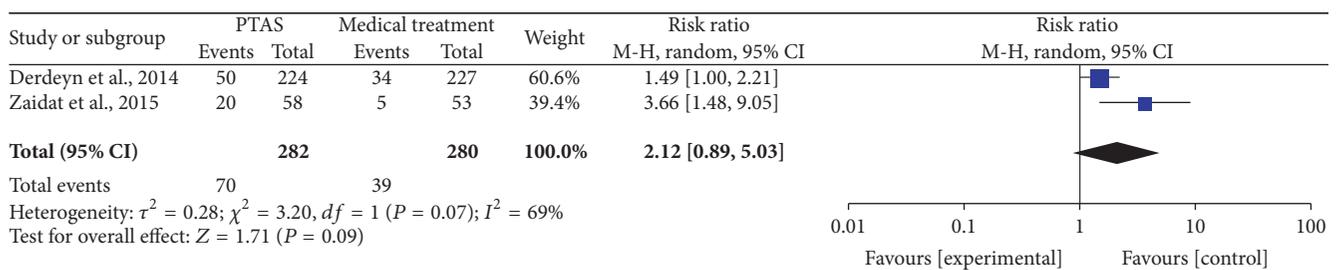


FIGURE 6: Forest plot of any stroke within 1 year for PTAS plus medical therapy versus medical therapy alone.

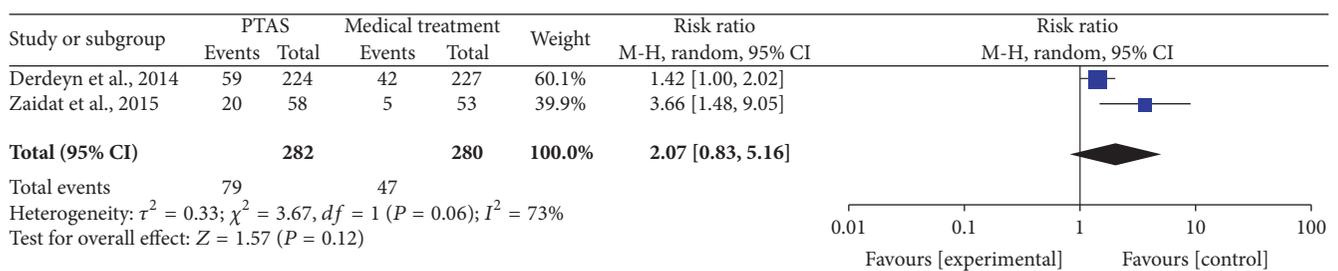


FIGURE 7: Forest plot of any stroke during the follow-up for PTAS plus medical therapy versus medical therapy alone.

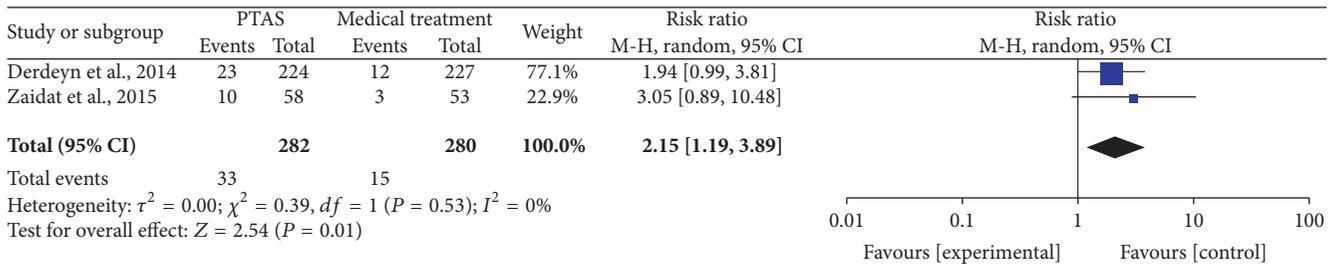


FIGURE 8: Forest plot of ischemic stroke within 30 days for PTAS plus medical therapy versus medical therapy alone.

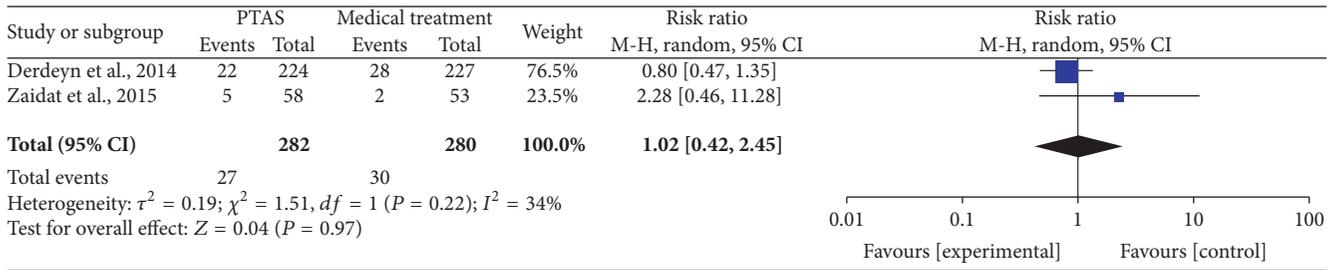


FIGURE 9: Forest plot of ischemic stroke beyond 30 days for PTAS plus medical therapy versus medical therapy alone.

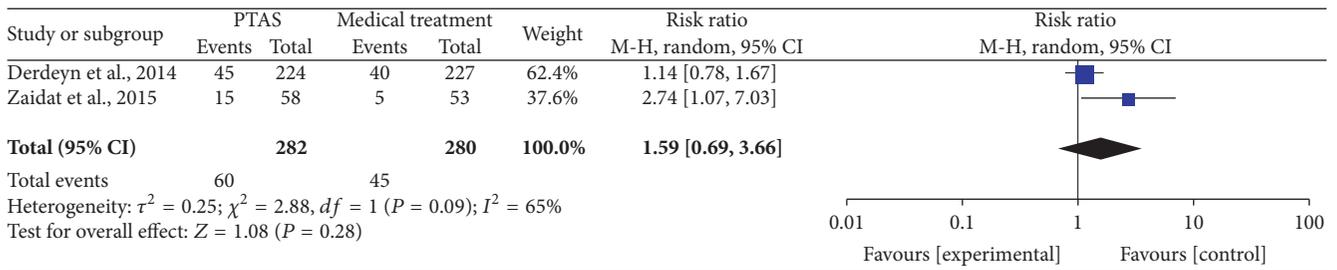


FIGURE 10: Forest plot of ischemic stroke during the follow-up for PTAS plus medical therapy versus medical therapy alone.

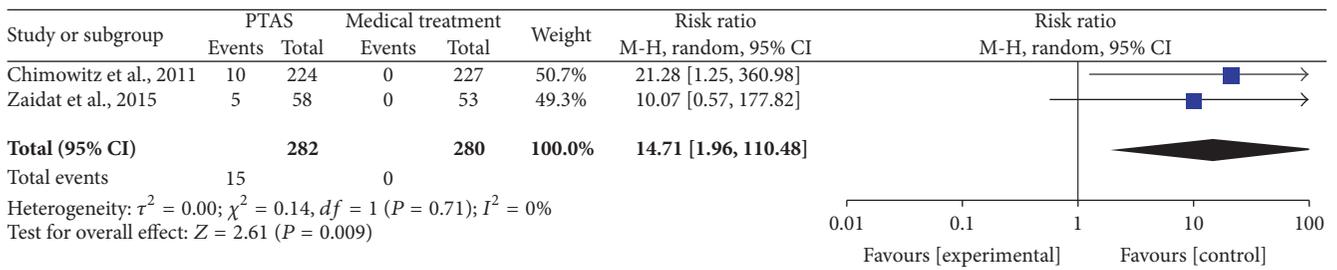


FIGURE 11: Forest plot of intracranial hemorrhage within 30 days for PTAS plus medical therapy versus medical therapy alone.

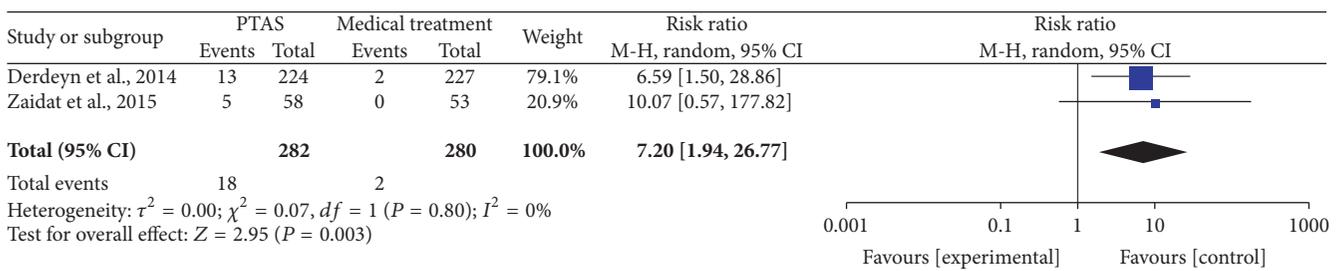


FIGURE 12: Forest plot of intracranial hemorrhage during follow-up for PTAS plus medical therapy versus medical therapy alone.

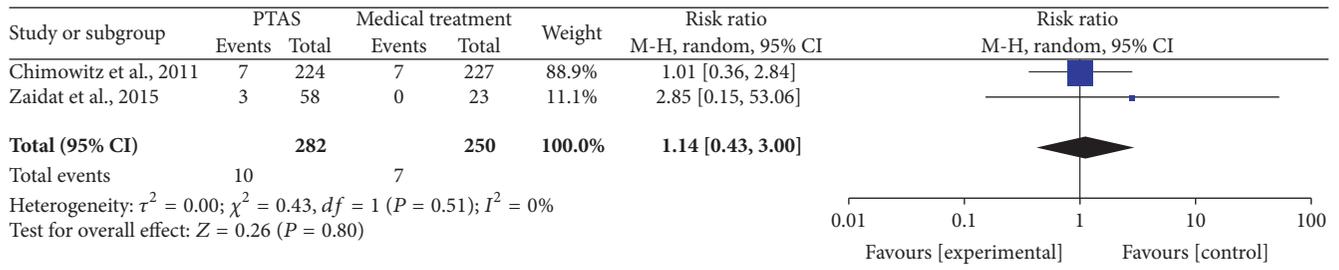


FIGURE 13: Forest plot of death within 30 days for PTAS plus medical therapy versus medical therapy alone.

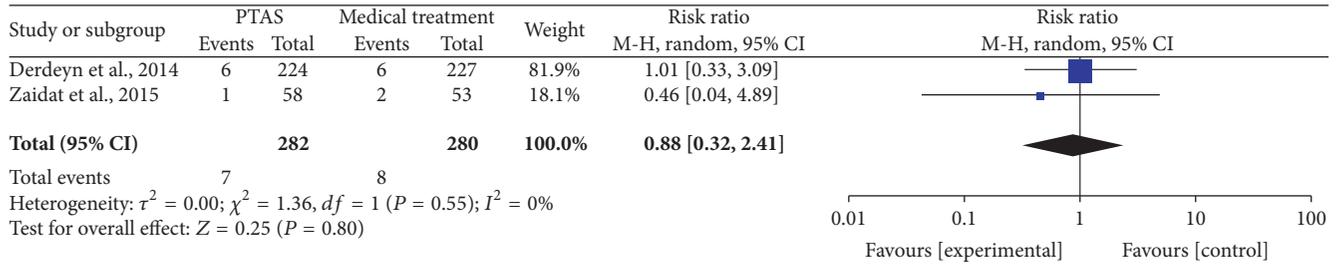


FIGURE 14: Forest plot of death beyond 30 days for PTAS plus medical therapy versus medical therapy alone.

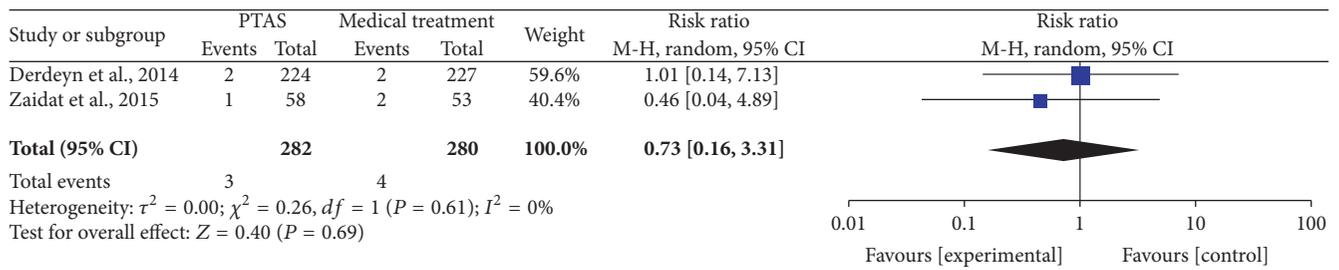


FIGURE 15: Forest plot of death between 30 days and 1 year for PTAS plus medical therapy versus medical therapy alone.

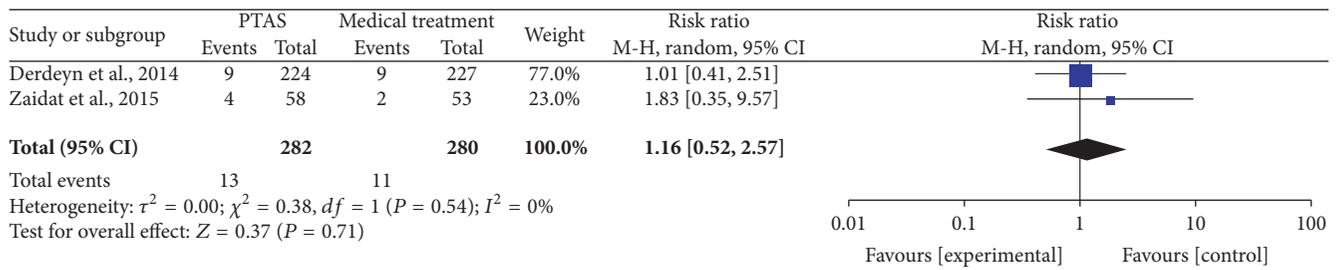


FIGURE 16: Forest plot of death within 1 year for PTAS plus medical therapy versus medical therapy alone.

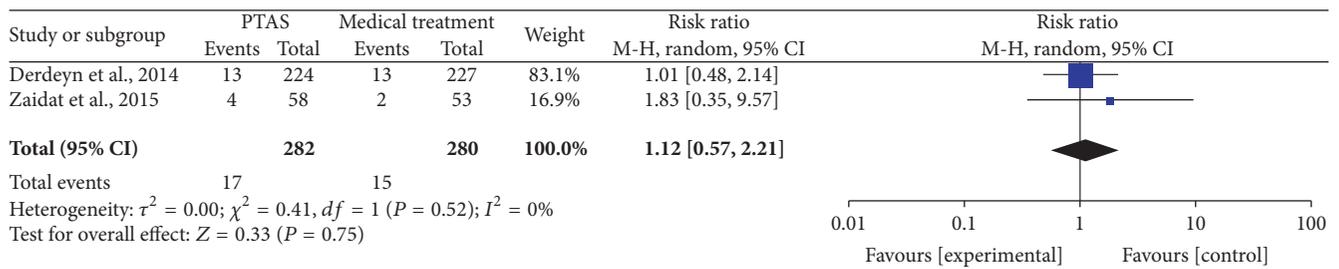


FIGURE 17: Forest plot of death during follow-up for PTAS plus medical therapy versus medical therapy alone.

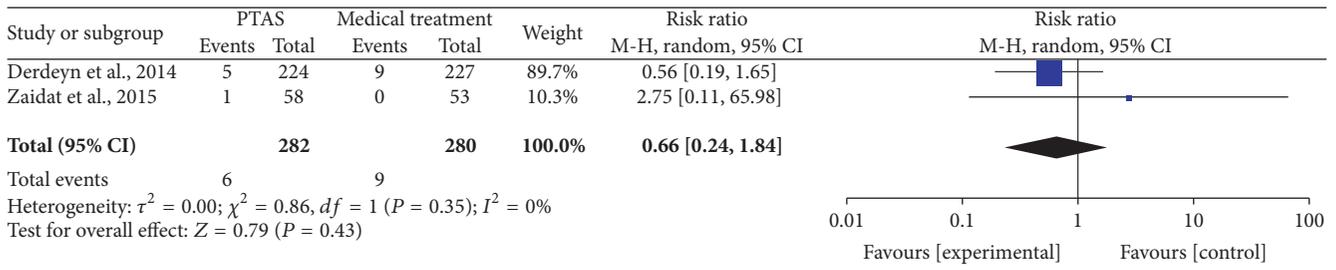


FIGURE 18: Forest plot of myocardial infarction during follow-up for PTAS plus medical therapy versus medical therapy alone.

likelihood of detecting superiority of stenting over medical therapy after 112 patients were randomized under the current trial design. The VISSIT trial was different from the SAMMPRIS trial in the type of stent (PHAROS™ Vitesse balloon-expandable neurovascular stent in VISSIT trial) but yielded similar outcomes to the SAMMPRIS trial [14], which indicated that the type of stent might not be related to the complications; and recent studies also suggested that the complication rates of balloon-expandable stents were similar to those of self-expanding stents [17–19]. The VAST enrolled patients who had vertebral artery stenosis of at least 50% in the Netherlands and this meta-analysis only selected patients with intracranial vertebral artery stenosis in this trial. VAST was stopped for some reasons and only 16 patients with intracranial vertebral artery stenosis were randomized. The 22% of patients (2/9 patients) with intracranial vertebral artery stenosis in the stenting group had a periprocedural vertebral artery stroke, which was the worst result in the three studies [15].

Meanwhile, any stroke, ischemic stroke, and intracranial hemorrhage during follow-up in medical therapy alone were lower, compared with PTAS plus medical therapy and the rate of periprocedural stroke after PTAS was higher than expected. The reasons could be as follows: the medical therapy has changed during the period of the above three studies, dual antiplatelet therapy became more common, the control of low density lipoprotein cholesterol and blood pressure became more strict, the intervention of life style became more important, and treatment began more timely. However, we could find that periprocedural complication was the main reason for the bad outcomes in PTAS group, and the lower the rate of periprocedural complication, the better the outcomes.

Recently several studies showed a lower rate of periprocedural complication in PTAS plus medical therapy for ICAD. Jiang et al. reviewed 637 patients with symptomatic ICAD at 5 high-volume centers (4 in the United States and 1 in China). The overall 30-day periprocedural complication rate was 6.1% [18]. Miao et al. recruited 158 patients with symptomatic ICAD caused by hypoperfusion combined with poor collateral flow and used tailored angioplasty and/or stenting. The 30-day rate of composite stroke, myocardial infarction, or death was 4.4% (7/158) [20]. Li et al. reviewed 433 consecutive patients with intracranial arteries stenosis $\geq 70\%$ and with symptomatic ischemic stroke or TIA (over

24 hours from the final TIA event and over 7 days from the final stroke) who underwent intracranial Wingspan stenting, and 30-day stroke rate was 6.7% (29/433) [21]. Miao et al. enrolled patients with TIA or stroke within the past 90 days due to hypoperfusion in the territory of the target ICAD and excluded patients with acute infarcts within 3 weeks. Tailored endovascular treatment of using balloon-mounted stent or balloon plus self-expanding stent for ICAD was based on anatomical features and lesion morphology. The 30-day rate of stroke, TIA, and death was 4.3% (13/300) [22]. Gao et al. enrolled patients with recent TIA or ischemic stroke related to high-grade stenosis of a major intracranial artery and with distal hypoperfusion and/or cortical involvement but excluded patients who had ischemic symptoms within the recent 3 weeks and perforator ischemic events. As a result, the overall 1-month stroke and/or death rate was 2% (2/100) [23]. Characteristics of the above trails were summarized in Table 2.

The low complication of the above studies might be related to the following reasons: investigators' experiences, patient selection, vascular morphology (lesions length, target vessel diameter, or vessel tortuosity, plaque positive and negative remodeling, and problem of perforator vessel), and the pathogenesis of ischemic stroke (perfusion deficits and longer time interval before the PTAS imply a stable plaque and may reduce the risk and complications [24, 25]). We thought that the pros and cons of these methods require a clear decision-making based on individual features. Effective exploration for PTAS is on the way forever, and the design of the studies should take imaging techniques, lesion features, stent type, different stages of ischemic stroke, and procedural techniques into consideration [26, 27]. There are many different endovascular treatments such as angioplasty plus low radial force self-expanding stent, balloon-expandable drug-eluting stent, high radial force self-expanding stent without angioplasty, and drug-eluting balloon with low or high radial force self-expanding stent. The improvement of stent might also provide the changes in efficacy. Undersized balloon angioplasty and deployment of an enterprise stent with reduced radial force have been proved safe and effective for intracranial stenosis [28, 29]. Neuroimaging is an important tool in clinical trials [30]. In recent years, there are more and more new techniques to help in directing the stent placement and secondary stroke prevention [31]. High-resolution magnetic resonance imaging (HRMRI) could display the features of arterial wall, which may be useful in identifying high-risk

TABLE 2: Characteristics of the trails mentioned in the Discussion.

Author/year	Cases	Trail detail	Eligible patients	Stenosis (% mean \pm SD)	Stenosis location	Stent type	Any stroke and death at 30 days (%)
Jiang et al./2012	637	Multicenter retrospective study of consecutive patients	Symptomatic ICAD	78 \pm 12	Intracranial ICA, MCA, BA, intradural VA	BES or SES	6.1
Miao et al./2015	158	Single center prospective cohort study	Symptomatic ICAD caused by hypoperfusion combined with poor collateral flow	82.01 \pm 7.43	Intracranial ICA, MCA, BA, intradural VA	BES for smooth access and Mori A lesion, SES for tortuous access and Mori B or C lesion, and angioplasty alone for tortuous access and Mori A lesion	4.4
Li et al./2015	433	Single center prospective study of consecutive patients	Over 24 hours from the final TIA event and over 7 days from the final stroke caused by ICAD	82.3 \pm 7.6	Intracranial ICA, MCA, BA, intradural VA	SES	6.7
Miao et al./2015	300	Multicenter prospective single-arm registry study	Symptomatic ICAD combined with poor collaterals and acute infarcts within 3 weeks were excluded	84.3 \pm 7.51	Intracranial ICA, MCA, BA, intradural VA	BES or SES	4.3
Gao et al./2016	100	Multicenter prospective single-arm trial	TIA or ischemic stroke caused by ICAD and ischemic symptoms within 3 weeks were excluded	82.7 \pm 8.9	Intracranial ICA, MCA, BA, intradural VA	SES	2

SD, standard deviation; ICAD, intracranial atherosclerotic disease; MCA, middle cerebral artery; VA, vertebral artery; ICA, internal carotid artery; BA, basilar artery BES, balloon-expandable stent; SES: self-expanding stent; TIA transient ischemic attack.

lesions for PTAS and selecting patients for intracranial PTAS [32–35].

In addition, the role and effect of PTAS may vary according to the different phases of ischemic stroke. In 2015, five RCTs have proved the efficacy of endovascular thrombectomy by using stentriever over standard medical care in patients with acute ischemic stroke caused by occlusion of arteries of the proximal anterior circulation, so endovascular thrombectomy has been recommend as the first-line method in recanalization therapy for large artery occlusion of acute anterior circulation [36–40]. Extending the time window of endovascular thrombectomy and improving reperfusion were important in acute ischemic stroke [41–43]. Based on these therapies, some physicians considered that PTAS can be used as a rescue treatment for failure of mechanical thrombectomy for large artery occlusion of anterior circulation [44].

The present meta-analysis still has some limitations. Only 3 eligible RCTs with 581 participants were included in this meta-analysis and the sample size is inadequate. In VAST with only 19 patients involved in meta-analysis for any stroke within 30 days, the publication bias might

exist. Moreover, although this meta-analysis had put equal emphasis on publications during literature search, there may be unpublished data beyond our search.

6. Conclusion

This meta-analysis demonstrated that any stroke and ischemic stroke in PTAS plus medical therapy occurred in early period after operation, and beyond 30 days the PTAS was not inferior compared with medical therapy alone. Periprocedural complication was the main reason for the bad outcomes in PTAS group, and the lower the rate of periprocedural complication, the better the outcomes. To reduce the rate of periprocedural complication, design of further studies should take imaging techniques, lesion features, stent type, different stages of ischemic stroke, and procedural techniques into consideration.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Zhong-Hao Li, Zhen-Hua Zhou, and Xian-Jin Zhu contributed equally to this work.

Acknowledgments

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Review Article

Preclinical Studies and Translational Applications of Intracerebral Hemorrhage

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Intracerebral hemorrhage (ICH) which refers to bleeding in the brain is a very deleterious condition with high mortality and disability rate. Surgery or conservative therapy remains the treatment option. Various studies have divided the disease process of ICH into primary and secondary injury, for which knowledge into these processes has yielded many preclinical and clinical treatment options. The aim of this review is to highlight some of the new experimental drugs as well as other treatment options like stem cell therapy, rehabilitation, and nanomedicine and mention some translational clinical applications that have been done with these treatment options.

1. Introduction

Intracerebral hemorrhage (ICH) is a devastating disease and the second leading cause of stroke [1]. It accounts for about 25 per 100,000 cases annually and results in high mortality rate [2]. ICH episode results in primary injury to the brain which initiates other devastating cascades leading to further damage. Treatment options still remain to be either surgical intervention or conservative therapy [3]. Primary injury occurs from direct injury by mass effect of the hematoma or by neurovascular disruption. Treatment during primary injury is still debatable between hematoma evacuation and conservative treatment, since at this point attempt to evacuate hematoma might lead to further brain damage [4]. Hematoma expansion further aggravates primary injury within first 24 hours of icterus. Secondary damage to the brain occurs due to series of events that are initiated by primary injury and its metabolites. These include insult from blood cell lysis (iron, heme, etc.), thrombin cascade activation, and inflammation [5]. Thrombin is implicated in the initiation of cerebral injury after hematoma [6] and

influences the degree of edema formation after ICH. Other studies have also identified thrombin to initiate blood-brain barrier (BBB) disruption [7], neuroinflammation, or neuroprotection at different quantities [8]. Different inflammatory cells and their mediators are involved in secondary damage. For example, mast cells and lymphocytes inhibition were seen to improve survival rate of experimental animals [9]. Inflammatory mediators like cytokines, matrix metalloproteinases (MMPs), and adhesion molecules have also been highlighted as causes of secondary brain injury [10–12]. Iron overload from lyses of red blood cells is another known factor of secondary injury, since it activates the ROS [13], leading to protein and DNA damage [14]. Disruption of the BBB allows proteins of complement system to easily cross into the brain. Crossing of activated complement cascade into the brain initiates the membrane attack complex (MAC) which further destroys the BBB allowing influx of fluid into the brain causing brain edema [15]. Perihematomal edema leads to mass effect and further aggravates secondary injury. Many clinical trials are therefore ongoing with some under completion, into different treatment options ideal for an ICH

patient. The essence of this review is to highlight some of the current preclinical studies into various treatment options for ICH, further stating some translational applications and clinical trials.

1.1. ICH Disease Process

1.1.1. Hematoma Enlargement. Enlargement of hematoma volume is seen to occur in about 30% of all patients [25], with about 72.9% of them having hematoma increase within the first 3 hours after incidence [26]. Hematoma may sometimes extend into the ventricles causing IVH (intraventricular hemorrhage) within first 24 hours after onset, in about 45% of all cases. A correlational study of IVH volume on prognosis predicted that a 2 ml increase in IVH within 24 hours indicated poor prognosis [27]. Other studies on the causes of hematoma expansion identified the shape of initial hematoma and the function of the liver to directly influence rebleeding. For example, Fujii et al. noted that patients with irregular shaped hematoma experience some degree of hematoma expansion compared to those with uniform shaped hematoma. They further noted that the same was true for patients with decreased fibrinogen and platelet count, as well as in patients with impair alpha 2-antiplasmin activity [28].

1.1.2. Brain Edema. Edema formation around hematoma is commonly seen within hours to days after ICH. Edema formation can be divided into 3 phases. The first phase is characterized by difference in hydrostatic pressure between retracted clot and surrounding brain tissue. This phase begins several hours after ICH and is then followed by activation of coagulation pathway and thrombin release. This is mark of the second phase which last about 2 days after ICH. During the third phase (after 3 days) there is vast release of hemoglobin from hemolysis of red blood cells. Activation of the complement system is therefore a major contributing factor to edema formation during the second and third phase [29]. The process of edema formation can therefore be summarized into the following events: that is, mass effect, clot retraction, thrombin formation, and hemolysis of RBC, hemoglobin release, complement system activation, and breakdown of BBB. Hoff and Xi therefore made an important accession that early evacuation of hematoma could interrupt the process of edema formation [30].

1.1.3. Neural Cell Death. Another devastating effect of ICH is neural cell death. During an ICH, reactive oxygen species (ROS) are released. These compounds initiate mitochondria-dependent or independent cell death pathways via oxidative stress [31]. Other studies have also identified that glutamate can affect the cell death. Glutamate production increases in brain parenchymal after an ICH episode triggering release of inflammatory cells and byproducts of erythrocyte breakdown. These products have been seen to be major free radical activators which in turn activate other cascades leading to cell death [32].

1.1.4. Inflammation. The presence of hematoma in brain parenchyma triggers brain's resident microglia. Activated microglia further recruits other leukocytes causing excessive release of inflammatory mediators [33]. Pozzilli et al. demonstrated that inflammation following ICH involves both resident and migration of circulatory cells to the brain [34]. Wu et al. also studied the effect of inflammation on edema formation reiterating the expression of macrophage inflammatory protein-2 (MIP-2) to contribute largely to edema formation. They added that MIP-2 expression began 2 hours and peaks 2 days after hemorrhage. Furthermore, they found that MIP-2 mediated edema formation was mediated by NF-kappaB activation [35]. Other studies found that a WBC count of 10,000/mL³ could lead to early neurological deterioration within the first 3 days of ICH [33]. Inflammation therefore plays a major role in disease process of ICH.

1.1.5. Recovery. After an ICH episode, some patients experience some extent of functional recovery although most patients live with some form of permanent disability. Many experiments have identified neurogenesis to account for the mechanism of recovery; Yang et al. found that thrombin formation can stimulate some amount of neurogenesis leading to some extent of functional recovery [36]. Similarly functional recovery is attributed to resolution of hematoma's mass effect, edema, and neuroplasticity of surrounding resident neurons [37].

2. Preclinical Studies of ICH: Models and Drugs

2.1. ICH Modeling. The constant tussle surrounding the treatment of ICH has called for many researches that seek to uncover the mystery behind the disease process. An ICH episode requires prompt attention and urgent intervention as such the study of ICH in the clinical setting is somewhat limited in patients. Studies on ICH could therefore be dependent on advanced imaging and other pathological studies. It is therefore prudent to model ICH in the attempt to mimic what happens in human patients. Till date there are three main techniques that are widely used in the study of ICH: autologous blood injection, collagenase blood injection, and microballoon injection (Table 1).

The collagenase injection model is the commonest model in recent times. This model is created by injecting about 0.075–0.4 U of collagenase into the basal ganglia of striatum of the animal allowing the collagenase to dissolve and rupture small vessels of the brain. It is therefore useful in simulating deep brain and penetrating vessel ruptured hemorrhages [38–40]. The collagenase injection model has the possibility of inducing cellular toxicity [38]. Therefore, more damage is done to basal ganglia putting in question its ability to correctly mimic the human ICH [41, 42]. The autologous blood injection model is another commonly used model. This model injects about 50–100 μ L of blood into the striatum or cortex of the rat brain. This was the earliest type of model used to replicate lobar hemorrhage and also to study the mechanism of brain injury in perihematomal region [43, 44].

TABLE 1: Common models for studying ICH.

ICH Models	Merits	Demerits
Autologous blood injection	Easy to perform Easy to reproduce Hematoma size fixed Mimics lobar hemorrhage	Needle trail reflux Cannot mimic rebleeding Short edema peak time
Collagenase injection	Mimics rebleeding and hematoma expansion No reflux needle trail Simulating perforating artery rupture	Possible cellular toxicity Inconsistent hemorrhage Excessive neural damage
Microballoon inflation	For studying mass effect Injury confined to inflation site	Minimal damage observed Only for simulating mass effect

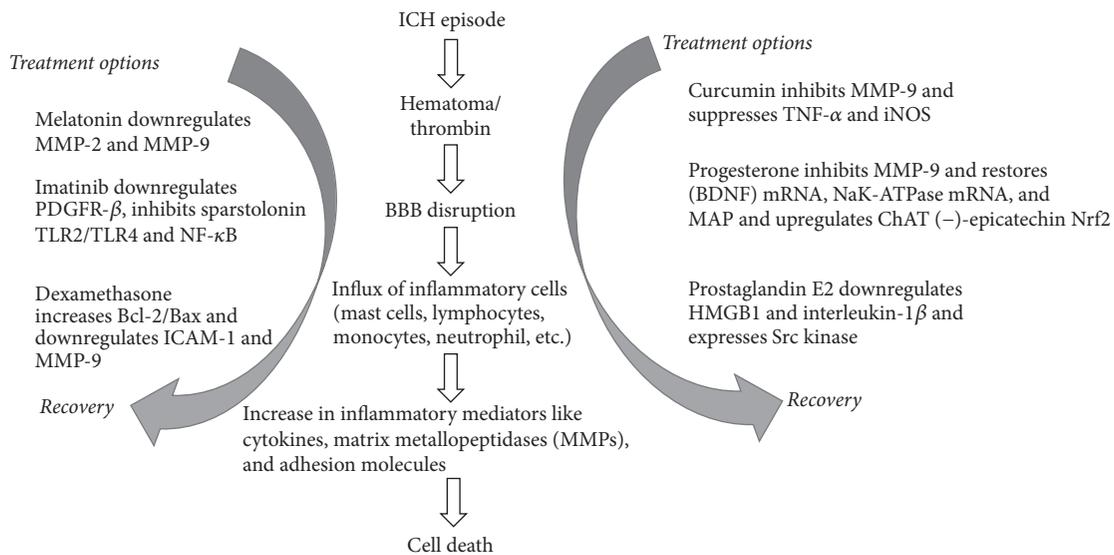


FIGURE 1: Preclinical drugs and their targets in ICH disease process.

This model is unsuitable for studying hematoma enlargement due to consistency of hematoma volume [40]. Another demerit of this method is the tendency of reflux of hematoma along needle trail. Another important setback is that excessive brain damage will occur during a speedy induction of blood [40, 45]. Mass effect was simulated and studied by Sinar et al., who inflated a microballoon into the rat’s brain. This model was used to find out the effect of hematoma evacuation on the brain [46]. The microballoon model can only simulate mass effect without putting into consideration the pathological effect of hematoma like edema. There is also minimal damage caused using this model making it impossible to truly mimic what happens in humans [47].

Animal models can basically be divided into the small and large animal model. With regard to advantages, the small animal model is cheaper due to cheaper cost of raising animals and is also easy to model. Small animals have short gestational period making experimental time shorter. It is therefore convenient for immunohistochemical and biochemical studies. The large animal model however presents the advantage of being close in anatomical structures and genetic composition to humans. They have large gyrus and well differentiated white matter making replication of

hematoma in large animal mimic humans more accurately. The replication of a successful small animal model in the large animal model will therefore potentiate the studies chances of being translated into the clinic.

2.2. Preclinical Drugs for Treating ICH. There have been many studies done on different drugs which have been proven in small animal models to be efficient in treating ICH (Figure 1). There is therefore the need to experiment more of these drugs in large animals to enable translation into clinical trials.

2.2.1. Treating Perihematomal Edema

Neuroinflammation and Oxidative Stress Drugs

Curcumin. Curcumin is a yellow pigmented polyphenol derivative of curcuma longa. It is widely used as coloring agent but has also been found to be useful in the treatment of various diseases [48]. Due to its antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer properties, curcumin has been studied as potential therapy for many infectious and cancerous diseases [49]. Curcumin is hypothesized to be useful in attenuating hematoma size

and reducing inflammation after hemorrhage. According to Fu and Kurzrock, curcumin influences biological processes either through direct impact on target protein or through epigenetic modulation, that is, the modulation of genetic environment without affecting gene structure [50]. Curcumin also possesses additional characteristics of being able to cross the BBB as well as having lower toxicity even at high dosages making it suitable for treatment of cerebral diseases. The optimum dose for maximum effect of curcumin was discovered by King et al. to be 150 mg/kg. King et al. in the same experiment found the therapeutic window of curcumin to be within 1–72 hours after injury with maximum efficacy within first 3 hrs [51]. Another study on curcumin's effect on ICH demonstrated its potential in attenuating edema via protection of BBB integrity after an ICH. The integrity of BBB is compromised during cerebral hemorrhage allowing for excessive influx of fluid and protein causing massive edema. Curcumin is able to inhibit the expression of MMP-9 and ionized calcium-binding adapter-1 (iba-1) positive microglia and strengthen the integrity of BBB by enhancing occludins and zonula occludens (ZO-1) expression [52, 53]. This effect will therefore lead to the inhibition of perihematomal edema and prevent further damage to the brain. Other studies on curcumin reported its function in reducing excessive inflammation and injury to the brain [54]. Curcumin treatment has also been seen to result in improvement in learning and memory functions after curcumin administration. This is due to curcumin's ability to suppress release of TNF- α and iNOS in the hippocampus consequently improving cognitive function [55]. Curcumin could therefore be used to reduce ICH related inflammation and edema and improve neurologic function. There is the need for further studies into its usage as a treatment option after ICH.

Progesterone. Progesterone is a steroid hormone with a well documented role in menstruation. An abnormality with this hormone therefore leads to menstrual abnormalities in female. Progesterone is produced by the ovaries but also believed to be a product of neurosteroids pregnenolone and dehydroepiandrosterone [56]. In animal studies, ICH deleterious effect on female animals was discovered to be somewhat masked due to the presence of progesterone. This raised the belief that progesterone could be a useful therapeutic option for ICH. Progesterone has been found to exert neuroprotective effect by attenuating inflammation and improving neurologic outcome. A study by Jiang et al., to determine the effect of progesterone on neurologic outcome, deduced from histopathologic results that progesterone reduces brain edema, decreases size of lesion, and attenuates neuroinflammatory activities. Furthermore, progesterone suppressed activity of MMP-9, carboxylation, and nitroxylation of proteins and suppressed other molecular inflammatory responses. The same study also discovered reduction in scarring tissue and brain tissue loss after progesterone application [57]. Similarly, the effect of progesterone on ICH was studied by Lei et al., who observed decreased edema formation, attenuation of neuroinflammation, and glial scar tissue formation after progesterone use. They also observed that these effects were heightened in male and

aged female rats but were less obvious in young female rats [58]. Progesterone also restores the normal functioning of brain-derived neurotrophic factor (BDNF) mRNA, Na-K-ATPase mRNA, microtubule-associated protein (MAP) 2, and choline acetyltransferase (ChAT) [59]. Progesterone therapy could therefore serve as treatment option for post-ICH edema and inflammation. Additional studies are therefore warranted.

(-)-Epicatechin. (-)-Epicatechin (EC) is a natural flavonoid molecule found in high concentrations in green tea and cocoa. Consumption of EC rich products has been identified to reduce blood pressure and the incidence of cardiopulmonary diseases [60]. EC has also been known for its use in the treatment of diabetes and other liver and heart diseases. EC can easily cross the BBB and is also capable of attenuating oxidative/reductive stress through NF-E2-related factor (Nrf) 2 pathway [61]. Nrf2 pathway has been regarded as a protective agent for many organs in the body [62]. It is able to activate tissue protective factors and antioxidant genes which alleviate tissue damage [63–65]. EC in an experiment by Lan et al. using a wild-type and Nrf2 knocked-out mice indicated an upregulation of nuclear accumulation and superoxide dismutase 1 (SOD1) in wild-type mice but no change in SOD1 for Nrf2 knockout (KO) mice. In addition, (-)-epicatechin treatment decreased HO1 expression in Nrf2 KO mice but with no change in wild-type. They therefore concluded that (-)-epicatechin treatment prevents brain damage via Nrf2 upregulation and activator protein-1 (AP-1) inhibition with AP-1 inhibition independent of Nrf2 pathway [61]. (-)-Epicatechin will therefore provide another treatment option in attenuating inflammation after ICH.

Prostaglandin E2. Prostaglandins are arachidonic acid derivatives. They are synthesized through the cyclooxygenase pathway and regulates the process of inflammation, immune response, bone resorption and red blood cell production [66]. Prostaglandin mediated inflammatory response has been implicated in the exacerbation of ICH. Zhao et al. identified prostaglandin E2 receptor EP1 to be involved in inflammatory response, with its inhibition yielding favorable functional outcomes in animal models [67]. Another interesting finding by Liu and Sharp suggested that Src kinase expression served as a double edged sword which when activated in the acute phase leads to the disruption of the BBB and subsequent increase in edema formation, while in the chronic phase it mediates repair of the BBB reducing edema formation [68]. Other experimental findings identified acute blockade of Src kinase by an antagonist, prevented formation of brain edema, and at the same time prevented the repair of BBB during continual blockage for 2–6 days. This is because continual blockade of Src kinase leads to the downregulation of brain microvascular endothelial cell (BMVECs) and perivascular astrocytes necessary for BBB repairs [7, 69, 70]. Misoprostol is an analog of prostaglandin E2 which is reported to attenuate cerebral injury within 24 hours following insult [71]. Misoprostol in an experiment was seen to decrease brain edema and neuroinflammation and improve functional outcome. This was achieved via the downregulation

of HMGB1, interleukin-1 β , and Src kinase expression. Furthermore, misoprostol alleviates inflammatory cascades and oxidative stress related brain injuries [72]. Prostaglandin's anti-inflammatory property is a needed quality in the fight against ICH; further studies are therefore a necessity.

Melatonin. Indoleamine melatonin is a hormone secreted by the pineal gland responsible for regulating sleep awake pattern and neuroendocrine processes [73]. Melatonin function is related to its ability to activate MT1 and MT2 receptors. Melatonin has been shown to reduce postischemic reperfusion related injuries like hemorrhages via downregulation of MMP-9 and MMP-2. Hemorrhages associated with postischemia could therefore be attenuated using melatonin treatment [74]. Lekic et al. also demonstrated that lower doses of melatonin at 1 and 24 hours for 3 days will lead to improvement of memory and striatal function after 8 weeks via neuroprotection and reduction of oxidative stress [75]. Although melatonin has effect on oxidative stress, its effect on brain edema formation is still under question. For instance, an experiment studying the antioxidative properties of melatonin revealed that melatonin had no effect on edema formation [76]. Melatonin has also been studied to improve electrical response to signal around hematoma region. Ueda et al. found the protection of oligodendrocytes and astrocytes via oxidative stress attenuation around hematoma region to be responsible for this behavior [77]. Melatonin therefore promises another alternative in the treatment of ICH and requires further studies.

Imatinib. Post-ICH inflammation is a common phenomenon after an ictus. Macrophages express platelet-derived growth factor receptor- β (PDGFR- β) and its agonist platelet-derived growth factor receptor-D (PDGFR-D) during ICH. This triggers an inflammatory cascade leading to further recruitment of other macrophages which causes neuroinflammation [78]. Inhibition of PDGFR- α has therefore been thought to be a therapeutic target for preventing inflammatory injury [43]. Imatinib a tyrosine-kinase inhibitor has been used over the years for the treatment of tumors and other bone malignancies. In neurological studies, imatinib has been studied to attenuate cerebral injury through inhibition of PDGFR- α [79]. In a research to validate the effect of imatinib on vasospasm, imatinib was seen to prevent vasospasm 24–72 hours via the downregulation of PDGFR- β , mitogen-activated protein kinase, TNC, and the deactivation of PDGFR [80]. Treatment of ICH with imatinib via inhibiting PDGFR promises to be an important therapy option that needs to be delved into.

Sparstolonin B (Toll-Like Receptor Inhibitor 4 (TLR4)). Toll-like receptors are pattern-recognizing receptors that recognize exogenous and endogenous molecular patterns initiating either adaptive or innate immunity [81, 82]. TLR4 has been implicated in inflammatory response after ICH. An upregulation of TLR4 has been reported in some studies with results indicating deterioration in condition after ictus in animal model [83]. Sparstolonin B (SsnB) is a *Sparganium stoloniferum* derivative which is studied to selectively inhibit

TLR2/TLR4 making it useful in the treatment of many inflammatory diseases [84]. Zhong et al. demonstrated that Sparstolonin B could serve as a suitable treatment for ICH. Their results indicated inhibition of TLR2/TLR4 heterodimer formation thereby inhibiting secondary injury after ICH. Activity of SsnB was however dosage dependent with higher dosage (50 μ mol/L) giving the highest efficacy. Furthermore, the same experiment indicated that SsnB inhibited NF- κ B activity via TLR2/TLR4 heterodimer formation giving indication of SsnB's capacity in attenuating inflammation [85]. SsnB could therefore be used as a neuroprotective agent to alleviate ICH related inflammation. Additional studies should be conducted to understand SsnB therapy in detail.

Dexamethasone. Dexamethasone treatment is used in the clinic for both spinal cord injuries and tumor to reduce edema formation [86]. Its application in the treatment of ICH has also received massive research. For instance, the effect of dexamethasone was studied in a rat model immediately after ICH and 3 days after ICH induction. The experimental results revealed drastic reduction in edema formation through increase in the Bcl-2/Bax ratio and downregulation of cleaved caspase-3 which are known indicators of inflammation in ICH [87]. Other studies have revealed the effect of DEX in treating edema via the regulation of intercellular adhesion molecule-1 (ICAM-1) and matrix metalloproteinase-9 (MMP-9) expression [88]. Other studies on the efficacy of different doses of DEX on edema proved that, even at smaller doses, dexamethasone was still able to attenuate edema formation. For instance, experimental studies to find out the effect of different doses of dexamethasone on edema resolution concluded that lower doses of DEX (1 mg/kg) had beneficial effect on brain edema [89]. DEX could therefore be used as a treatment drug for attenuating inflammation and edema after an ICH episode. Further studies on the mechanism of action, including side effects, are needed.

2.2.2. Treating Hematoma Growth

Plasma Kallikrein Inhibitor (Aprotinin). Plasma kallikrein-kinin system (KKS) is made up of proteins factor XII (FXII), prekallikrein, and kininogen. The function of KKS includes regulating blood pressure, angiogenesis, inflammation, and cell proliferation and death. KKS is also strongly associated with coagulation, fibrinolysis, and vascular permeability. Experimental studies of KKS showed increased permeability of BBB accompanied by cerebral edema [90]. KKS activation increases vascular permeability and leads to blood extravasation from capillary causing hematoma enlargement [91]. Plasma kallikrein (PK) has been studied to increase hematoma volume in hyperglycemic rat model. Activation of PK affects collagen induced aggregation of platelets but has no effect on thrombin induced pathway [92]. A different study on the effect of prekallikrein on hematoma expansion after tPA treatment further stressed on the effect of plasma kallikrein (PK) on rebleeding. Thus the study concluded that inhibition of PK could serve as a therapeutic target to control rebleeding associated with tPA usage [93]. Aprotinin is a Kunitz-type

protein and a known inhibitor of trypsin, plasmin, and both tissue and plasma kallikrein. Effect of aprotinin and its recombinant variant form on hematoma enlargement was done in rat subdural hematoma model. Experimental results showed that decrease in plasma kallikrein reduced vascular permeability and blood extravasations thereby reducing perihematomal edema and hematoma enlargement [94]. The use of aprotinin to reduce rebleeding is a possible therapy for ICH. Till date not many experiments have been done to explore the PK inhibitor as a treatment target of ICH; further studies are therefore needed.

2.3. Other Preclinical Therapies

2.3.1. Neuroprotection and Functional Recovery

Nanomedicine. Nanomedicine refers to the use of nanoparticles in the treatment of disease. Several studies on the use of nanotechnology in the treatment of neurological disease have been conducted. The primary advantage of nanoparticles as carriers is to prevent the peripheral toxicity. The function of mitochondria is determined by the microviscosity of its membrane, which is affected by ischemic injury. In an experiment nanocapsulated-quercetin was seen to give protective effect to the mitochondria wall. The effect of nanocapsulated-quercetin on inducible nitric oxide synthase (iNOS) was further studied by Ghosh et al. who indicated that oral nanocapsulated-quercetin was able to downregulate iNOS which is expressed in ischemic cells compared to free quercetin administration. This further proved that nanocapsulated agents presented with efficacy and specificity as compared to their respective free agents [95]. In another experiment to demonstrate the effect of free quercetin as against nanoloaded quercetin's effect after ICH, nanocapsulated-quercetin proved better efficacy in reducing hematoma size, preserving glutathione S-transferase (GST) activity, and exhibiting total antioxidant properties [96]. Rapid functional recovery was therefore seen in nanocapsulated alarming researchers on the probability of using nanoparticles as treatment option for ICH. The use of self-assembling nanoparticles (SANP) in the treatment of ICH was also studied. This study identified SANP to reduce cavity formed after hematoma resolution and also improve functional recovery. SANP was seen to be compatible with local brain tissues and was therefore able to be transplanted into hematoma region reducing brain cavity [97]. The neuroprotective effect of poly(lactic-co-glycolic) acid (PLGA) nanoparticles loaded with recombinant human erythropoietin (rhEPO) was studied in hemorrhagic stroke model. The results indicated some level of functional recovery with decrease in brain damage [98]. Similar effect was seen in another experiment by Balabanian et al. who found improvement in rat model after rhEPO-loaded PLGA [99]. Nanoparticles application in the treatment of ICH presents a promising treatment option that should be looked at.

2.3.2. Treating Neural Death and Neuroinflammation

Enhancing Endogenous Neurogenesis. The subventricular zone produces most of the neural cells that migrate along olfactory

bulb. The extent of migration is however dependant on the presence of focal injury. Various experiments have shown that during injury SVZ cells migrate to the injured site and differentiate into glial cells [100, 101]. It is on this basis that many researchers have focused on how to initiate neurogenesis and increase migration of SVZ cells to treat neurogenic diseases. Many different pathways have therefore been researched into with the aim of initiating neurogenesis for which activation of macrophage/microglia seems to be one. In vitro study by Walton et al. found microglia to play significant role in neurogenesis. Their study indicated that activated microglia produces necessary factors that induce neurogenesis but do not induce neural cell proliferation themselves [102]. Another study about the role of microglia and T-lymphocytes in inducing neurogenesis discovered that, in an immune deficient model, neurogenesis was impaired even in a conducive microenvironment, further stressing on the importance of inflammation in neurogenesis [103]. Similarly, activated microglia turns to inhibit the expression of TGF-beta 1 an endothelial proliferation inhibitor in normal brain and upregulate TNF which is an angiogenic factor in injured brain [104]. This is due to the release of proangiogenesis factors VEGF and IL-8 by activated microglia. Angiogenesis could also be induced by neurotrophic, growth factors, anti-inflammatory drugs, hormones, and noncoding RNA. For instance, intrastriatal injection of glial cell line-derived neurotrophic factor (GDNF) has been seen to induce neurogenesis after ischemia [105]. Similarly, IGF-1 and GDNF have been seen to extend the survival time of progenitor cells [106]. Other agents including indomethacin and erythropoietin have been used to initiate neurogenesis and angiogenesis [107, 108]. Chemokines such as monocyte chemoattractant protein-1 (MCP-1) can also induce migration of SVZ cells to damaged regions [109]. After an ICH ictus, there is an upregulation of trophic factors like VEGF, hypoxia-inducible factor-1 α , Ang-1, and Ang-2. These factors will therefore promote some degree of angiogenesis in the brain. Another important compound that induces angiogenesis in hemorrhagic brain is thrombin. Thrombin is studied to induce angiogenesis by activating these trophic factors [110]. Enhancing endogenous neurogenesis and angiogenesis promises to be a novel therapy to improve functional recovery and reduce brain damage. Further studies should therefore be conducted in that respect.

Cell Transplantation. Stem cells transplantation has been studied in the treatment of many diseases. Currently, cells being used for preclinical studies include neural stem cell (NSC) or progenitor cells [111], immortalized cells [112], and human mesenchymal stem cells (hMSCs) and human bone marrow stromal cells (HBMSCs) [113]. Neural stem cells therapeutic effect has been clarified by many researchers. These studies focused on how the application of neural stem cells could improve functional recovery and the mechanism of action. One such experiment considered the ability of NSC to produce superoxide dismutase (SOD1) to override ROS stress. Similarly, intravenously transplanted NSC was seen to improve neurologic function [114]. These transplanted cells are able to differentiate into either neurons or glial

cell to replace damaged cells [115]. Another preclinical studies on NSC identified the ability of transplanted NSC to reduce neuroinflammation via the downregulation of gamma delta T cells and inflammatory markers but increases in anti-inflammatory markers and regulatory T (Treg) [116]. Mesenchymal stem cells (MSC) have also been intensely studied. The effect of MSC on various stages of ICH has thus been illustrated. For instance, the effect of MSC on BBB protection of ICH was seen to be enormous, in that MSC prevents BBB disruption via upregulation of TNF- α stimulated gene/protein 6 (TSG-6). MSC also strengthens the effect of zona occludens-1 and claudin-5 which are integral parts of BBB structure. Another experiment treating ICH with a combination of human mesenchymal stem cell and minimally invasive evacuation recorded improvement of functional recovery [117]. Muse cells are nontumor pluripotent stem cells that have been experimented to be potent in mouse ICH model. Experimental results with implanted muse in mouse model indicated functional recovery models as well as positive test for NeuN and MMP-2. The muse cells were found to be firmly implanted in the mouse brain and differentiated well into neural cells to help functional recovery [118]. Another promising field that has gained attention in recent times is the implantation of induced neurons which are from the cellular reprogramming of fibroblast cells into induced pluripotent stem cells [119]. To get a more efficient protocol for generating neurons from fibroblast that can survive implantation, Pereira et al. delayed that activation of transgene after viral transduction and further treated them with SMAD signaling downregulation and WNT signaling activation molecules. This protocol led to enhancement of neural survival [120]. Similarly, when noggin and SB431542, two inhibitors of SMAD signaling, were inhibited, there was report of conversion of human embryonic cells and iPS [121]. Mesenchymal stem cell from umbilical origin has been found to reduce edema formation and further brain damage when implanted in the 4-day postnatal model. This was done to investigate if mesenchymal stem cell transplantation will offer a treatment option for the very challenging treatment of IVH in premature infants [122]. Cell transplantation therefore presents a promising field which should be explored.

3. Translational Therapeutic Drugs

3.1. Treating Perihematomal Edema. Brain edema is a frequent occurrence after ICH. The effect of edema cannot be underestimated since it leads to mass effect which further aggravates brain damage which results in death [123]. The process of edema formation has been explained by many different mechanisms with some postulating edema to be caused by imbalance in oncotic pressure or result from BBB disruption by inflammation. The process of edema formation can be categorized into 3 phases. Edema formation in early hours of ICH is attributed to hydrostatic pressure, clot dissolution, and displacement of hematoma serum into the surrounding [124]. The second phase results from inflammatory cascade and thrombin formation, whereas the third phase arises from hemoglobin toxicity due to red blood cell

breakdown [123]. Understanding of these mechanisms has led to the development of some potential therapeutic drugs with some currently being used in the clinic as seen in Table 3.

3.1.1. Osmotherapy

Mannitol. Mannitol is a commonly used agent in the clinic for the treatment of edema. The efficacy in treating post ICH edema is still controversial. An experiment with five patients highlighted benefit of mannitol in improving clinical outcome [16]. A clinical trial with 2839 acute ICH patients, however, identified mannitol to have minimal significance in ICH recovery [125]. Other additional trials should therefore be done to establish the accurate effect of mannitol on ICH.

Hypertonic Saline. Hypertonic solution is another well-known agent for osmotherapy. In a canine ICH model, hypertonic saline was seen to reduce intraparenchymal pressure difference that occurs during ICH with effect lasting for about 3 hours [126] which inevitably leads to the control of edema [126]. Hypertonic saline (23.4%) is seen to control ICP and subsequent herniation of the brain [127, 128]. A retrospective study of the effect of three percent hypertonic saline on ICP and recovery showed the use of hypertonic saline as a feasible treatment of edema and ICP after severe ICH [129].

3.1.2. Neuroinflammation and Oxidative Stress Drugs. After an ICH episode, inflammatory cascade is triggered. This begins with the initial damage of tissues and then the activation of neuroinflammatory factors. Inflammatory factors in turn lead to the disruption of blood-brain barrier. After the integrity of BBB is compromised, circulatory inflammatory factors are able to cross the brain causing further tissue damage which in turn also activates other factors that initiate tissue repair and subsequently lead to recovery [130]. Many studies have therefore targeted the alleviation of inflammation as a novel treatment for ICH [131].

Celecoxib. Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor. In some animal experiments its mechanism of action has been seen to be effective in anti-inflammation, antioxidation, and neuroprotection. An experiment by Chu et al. suggested that the inhibition of prostaglandin E was probably the reason behind celecoxib's therapeutic property [132]. Smaller randomized control trials (RCT) have been done with patients treated with celecoxib. The result of such study which treated patients with 400 mg/kg of celecoxib for about 7 days showed reduction in hematoma and edema volume, further stressing the safety and efficacy of this drug [133]. Another small trial also stressed on the edema attenuating effect of celecoxib administration. Celecoxib should therefore warrant further larger studies into its application for use in ICH treatment [134].

Fingolimod. Fingolimod (FTY720) is a sphingosine-1-phosphate receptor (S1PR) modulator which has received many studies into its role in ICH treatment. The outcomes of these studies have been debatable between those indicating positive outcome and those showing no benefit after acute

ICH [135]. Lu et al.'s experiment on the neuroprotective effect of fingolimod is one of such studies which concluded that brain atrophy and neuroinflammation are significantly reduced following fingolimod administration resulting in improvement of neurological function [136]. Another experiment demonstrated that fingolimod significantly lowered lymphocyte count in experimental animals as well as intercellular adhesion molecule-1 (ICAM-1), interferon- γ (INF- γ), and interleukin-17 (IL-17) count in experimental model [137]. Similarly, a 2-arm clinical trial study of oral fingolimod's effect on perihematomal edema was conducted by Fu et al. In their study, fingolimod was seen to alleviate edema formation and inflammation and improve neurologic outcome [18]. Although fingolimod has protective properties there are also some harmful effects associated with its usage. One way of improving its usage is the use of RP101075 an S1PR modulator agonist with less cardiotoxicity [138]. Fingolimod therefore needs further studies to potentiate its efficacy and reduce its adverse effect in humans.

NXY-059 (Disufenton Sodium). Disufenton sodium is a nitrene with the ability to spin trap free radicals [139]. Due to their carbon-nitrogen bonds, they are able to bind to reactive radical, stabilize them, and prevent them from destroying cell [140]. Many studies with animal model have identified its beneficial effect. For example, studies have identified the therapeutic window for transient and permanent stroke was 2 hrs and 240 mins, respectively, after incidence [141]. Another study indicated that when NXY-059 was used immediately after an embolic ischemia, hemorrhage was likely to occur due to its effect on cerebral vasculature. NXY-059 in combination with tPA however reduced tPA-induced hemorrhage [142]. Similarly, NXY-059G has been seen to have neuroprotective properties and therefore leads to functional recovery when administered [143]. The treatment benefit of NXY-059 was also studied in ICH models. Although rat models showed increase in neurological functions with lower neutrophil infiltration in perihematoma regions, there was no difference between hematoma size for models and control [144]. NXY-059 has undergone different phases of clinical trials. The CHANT phase I trial established NXY-059 as a safe and well tolerated drug for ICH especially at the acute phase within 6 hours [19]. Although well tolerated a trial by Strid et al. indicated that for patients with renal impairment, dosages should be adjusted since nonrenal clearance of NXY-059 is insignificant [145]. Further research into NXY-059 will therefore be helpful in treatment of ICH.

3.1.3. Iron Chelator

Deferoxamine Mesylate. Deferoxamine mesylate is an iron chelator approved for detoxication during acute or chronic iron overload [146]. Deferoxamine reduces hemoglobin induced edema, regresses brain atrophy, and improves neurological deficit in animal model [147]. Deferoxamine also reduces the rate of hematoma clearance and affects endogenous ICH response [148]. The dissolution of a hematoma

results in the creation of a cavity at the hematoma region. Deferoxamine in a study was found to reduce the size of cavity created after clot resolution. Also, the effect on cells with ferritin and HO-1 present was significantly reduced [149]. Deferoxamine has gone through the phase I trial to determine the maximum tolerated dosage. A dosage of 7 to 62 mg/kg/day was given which was well tolerated with some of the candidates experiencing some adverse effect which is not related to drug [150]. Clinical trials (NCT02175225 and NCT02367248) are currently ongoing to establish the effect of deferoxamine on perihematomal edema.

3.2. Treating Hematoma Growth. Rebleeding after an ICH is a common phenomenon which is also a common predictor of outcome after ICH [26]. It is estimated that about 30% of patients will experience rebleeding during early hours of being hospitalized [26]. The treatment of hematoma regrowth could therefore be a surrogate target for ICH treatment (Table 3).

3.2.1. Homeostasis. Recombinant factor VIIa (rFVIIa) has been used for the treatment of blood related disorders like hemophilia and congenital factor VII deficiency [151]. Many experiments have been done to study the effect of this factor on ICH. This is to say, will the administration of Recombinant factor VIIa (rFVIIa) have any effect on hematoma size and if so what effect? In a 90-day RCT study of the effect of Recombinant factor VIIa (rFVIIa) on hematoma size, rFVIIa was seen to reduce the size of hematoma in experimental group as compared to the placebo group [17]. The therapeutic effect of rFVIIa also seems to be dose dependent, with higher doses recording higher efficacy and resulting in some degree of functional recovery in the same group [152].

3.2.2. Anti-Platelet Treatment Reversal. In clinical setting, most patients with ICH have been confirmed to be on at least one antiplatelet medication prior to admission. Antiplatelet has therefore been implicated as a contributing factor of ICH. In an animal model to study the effect of antiplatelet on ICH, it was discovered that there was no significant difference between the pretreated and control group [153]. This is not the case in humans since studies have indicated risk of ICH increases with antiplatelet usage. For example, clopidogrel or ticlopidine usage is seen to have higher risk of ICH than use of aspirin [154]. The reversal of antiplatelet treatment has therefore been controversial although there are some data supporting good outcome after reversal [154, 155]. The third phase of a clinical trial about the effect of platelet administration to patient on antiplatelet therapy indicated that platelet administration was of no much benefit to these patients [156]. Currently, the Neurocritical Care Society treatment guideline recommends desmopressin (0.4 mg/kg IV) on admission and platelet administration for preoperative preparation [157].

3.2.3. Blood Pressure Control. Hypertension with a systolic pressure of BP \geq 140/90 mmHg is seen to be the major cause of ICH in about 70% of all cases and also an indication of

poor prognosis [158]. The effect of hypertension on post-ICH outcome has therefore been studied in many models. For instance, Sang et al. noted in their experiment that although BP did not lead to spontaneous stroke during their period of study, there was some level of degeneration observed in ICH models despite higher neural stem cell (NSC) recruitment [159]. The impact of lowering blood pressure on hematoma growth has been studied in various trials. For instance, the INTERACT trials identified intensively lowering BP to about 140 mmHg resulting in decrease in chances of rebleeding [21]. Another trial, ADAPT trials (NCT00963976), also focused on acutely lowering BP and its effect on hemodynamic blood flow to the brain and also on hematoma growth [22]. ICH induces a transient disturbance in sympathetic system. As such some therapeutic targets have sort to find the effect of antiadrenergic drugs in the treatment of post-ICH. In CHANT's trial of 303 patients, antihypertensive drugs were associated with decreased edema after BP and hematoma size control [160]. Similarly, other studies have further highlighted the impact of antiadrenergic on the ICH. Another experiment indicated that β -blockers, that is, atenolol, slightly improve neurologic outcome after ICH and also prevent complications like pneumonia or SIRS after ICH [161]. Other conflicting trials however indicated that there was no much difference in outcome between various antihypertensive drugs [162]. Another example is the ATTACH trial which found no significant evidence to support effect of lowering BP on hematoma growth and perihematoma edema but encouraged other trials into the effect of aggressive BP lowering on ICH [23].

3.2.4. Hematoma Resolution

Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ). PPAR γ and its agonist have been instrumental in the treatment of metabolism disorders of glucose and lipid [163, 164]. However, PPAR γ has more recently been implicated in the attenuation of inflammatory, oxidative, and excessive phagocytic processes giving indications of its possible use in stroke and ICH treatment. A research by Zhao et al. hypothesized that PPAR γ could improve clot resolution by upregulating the phagocyte activity of microglia through CD36 regulation. Activation of the microglia cells will lead to faster uptake of red blood cells and quicker resolution of hematoma [165]. The role of PPAR γ in anti-inflammation and antioxidation has also been studied. PPAR γ has been found to increase the expression of anti-inflammatory cytokines TGF- β and IL-10 and also antioxidative enzymes catalase and superoxide dismutase [166, 167]. Preclinical studies have proven very positive with results indicating PPAR γ 's ability to improve clot resolution and provide neuroprotection while improving neurological function [168]. Clinical trials with PPAR γ agonist Pioglitazone were started on the safety of Pioglitazone in hematoma and edema resolution [20]. The second phase of the same experiment indicated safety of PPAR γ in humans for hematoma resolution (NCT00827892) [20].

3.3. Treating Neural Death. The use of stem cell for treatment of various diseases has received major boost in research and clinical trials (Table 3). Stem cells have the ability to differentiate into multiple cells hence their exploration for the treatment of disease like ICH. Treatment focuses either on enhancing longevity and production of endogenous stem cells from the subventricular zone and dentate gyrus or on exogenous transplantation of cells from other sources preferably neural or bone marrow origin [169, 170]. Although cell therapy has demonstrated some treatment benefit in animal model, its application in the clinical setting is still incomplete. Suarez-Monteagudo et al. studied the safety of implanting autologous bone marrow stem cell (BMSC) into perihematoma sequel after 12 months of ICH and stroke. They concluded that BMSC could be well tolerated by patients with no complication [171]. Another study revealed that when autologous bone marrow mononuclear cells were implanted through drainage tube, some degree of neurological function recovery was attained [172]. Similarly, the safety of autologous mesenchymal stem cells (MSC) was tested by Bhasin et al., who concluded that MSC was safe for implantation [173]. Intrathecal administration of bone marrow mononuclear cells (BMMNC) was seen to improve ambulatory function especially for young patient and patient with lesser duration of stroke incidence. This trial (NCT02065778) done on 24 patients after BMMNC was implanted intrathecally and followed up after 4 days for adverse effect and 6 months to 4.5 years for functional recovery [24]. Zhu et al. also performed another trial combining surgery with injection of BMSC, that is, through drainage tube and follow-up with intrathecal injection. A follow-up of treatment showed safety in treatment and decrease in National Institute Stroke Scale (NIHSS) and Rankin scale but increase in Barthel index [174]. There are currently 3 ongoing trials ((NCT02245698/India), (NCT01832428/India), and (NCT01714167/China)) on stem cell therapy which will present a major milestone in the treatment of brain injury through ICH or ischemia.

4. Improving Functional Recovery

4.1. Prosthetic and Robotic Therapy. One devastating effect of ICH is the impairment of neurological or motor function. The use of prosthesis to enhance movement of the paralytic side has also received some attention. The idea is for the prosthetic part to give support and offer some amount of coordination. Recently, many studies have been done to compare the use of prosthetic training against traditional treadmill gait training. One of such studies revealed significant difference between kinetic abilities at the paretic side for prosthetic part as compared to the treadmill training. It revealed that prosthetic body part combined with treadmill training resulted in early recovery [175]. In another study, the effect of neuroprosthesis on a cerebral palsy child was conducted. The researchers identified some degree of improvement in motor functions as the prosthetic body parts were worn over time suggesting the essence of time in recovery [176]. In other clinical setting, the use of robotic therapy together with conventional therapy showed improvement in motor functions. Robotic parts may

serve as a support for plegic side of patients [177]. Similarly, a research by Mehrholz et al. indicated that when paretic patients were given electromechanical and robot-assisted arm, motor function of the hand improved. This was because the robotic part takes up a surrogate role for the plegic hand. They however also stated that care should be taken when interpreting results due to discrepancies in duration intensity in the training for different test subject [178]. Robot-assisted training will therefore serve as a novel treatment for improving motor impairment and improving quality of life [179].

4.2. Brain-Computer Interface or Neuroprosthesis. Over the years scientists are trying to find ways to convert electrophysiological waves of the brain into messages that will be used to communicate with the environment [180]. This has led to the development of brain-computer interface (BCI). BCI uses five basic brain impulses, namely, visual evoked potentials, slow cortical potentials, cortical neuronal activity, beta and mu rhythms, and event-related potentials [181]. In patient with severe paralysis (locked in) there is some difficulty in communicating with the environment, although there is some amount of brain activity. The conversion of brains EEG into message which could be understood is therefore a priority of BCI. Different BCI's differ in signal collection, translation of signal, and relay of the information to the user. This disparity makes comparison between different laboratories somewhat difficult if not impossible. As a result, Kübler and Neumann reviewed BCI2000 an online BCI which allows for comparison of different BCI from different laboratory. Their interface differed from other BCI in that it processes neural information online which provide a less costly and effortless access to information data as compared to individualizing BCI [182]. Another study indicated that a locked in syndrome patient due to brain stem stroke can benefit from noninvasive visual P300 speller to enhance communication. This could therefore prove to be of equal benefit to patients who suffer severe paralyse from ICH [183]. There is therefore the need to study more this modality to enable patient with severe paralyse from ICH a chance to communicate with the environment.

4.3. Electro-Acupuncture. Traditional Chinese medicine has been practiced for over thousands of years in the treatment of many diseases of which stroke is part [184]. Meta-analytical studies revealed different approaches employed for stroke treatment, with varying results from improvement, adverse reactions to treatment, and sometimes even death [185]. In recent years, scientists have tried to blend the use of TCM with other scientific methods. For instance, Zhao and Yu explored the effect of cranial acupuncture on serum IL-6 content. They reported an improvement in nervous function and decrease in serum IL-6 [186]. Electroacupuncture (EA) is the combination of acupuncture and electrical stimulation. EA has been identified to attenuate the disruption of BBB after stroke. In a rat model experiment to examine the effect of EA on BBB, Wu et al. used Evans blue dye as a marker of extent of BBB disruption. They concluded that EA has

BBB protective ability [187]. EA at acupoints "GV26" and "GV20" for 30 min has been found to have an antioxidant property in rat model. Results from Zhong et al. experiment showed improvement in mitochondrial function accompanied with succinic dehydrogenase, NADH dehydrogenase, and cytochrome C oxidase activity increase. Results therefore indicated an elevation respiratory enzymes activity and a decrease in reactive oxygen species (ROS) and production [188]. The influence of EA on growth factors has also been studied. EA has been seen to increase cerebral blood flow [189]. Although some amounts of clinical studies have been done on EA (Table 3), the evaluations of results have been moderate [190]. The need to carry out further accurate trials and data collection is needed in ensuring the practicality of EA in ICH treatment.

5. Limitations of Translating Preclinical Studies

Preclinical animal model studies have enabled us to understand ICH disease process to some extent leading to some amount of translational application. With our current understanding of ICH, conservative treatment could be clinically targeted at different stages of the condition as shown in Figure 2. However, there is more to the treatment of ICH than the scope of this review. There are still some limitations that are yet to be overcome to propel these preclinical studies into clinical usage. Till date, the creation of an ideal model that fully mimics the entire disease process of ICH in humans is still a challenge. Most of the current models fail to develop a model that fully incorporates epidemiological and nonepidemiological factors of ICH. Another challenging factor is minimizing human or experimental errors. Although most of the preclinical drugs have experienced some level of success during experimentations in ICH models, these drugs are yet to be experimented in large animals or humans. Although some of these experimental drugs have already been used in clinical trials in the treatment of other diseases their advancement into ICH related clinical trial is yet to receive a breakthrough. This is due lack of drug specificity and other deleterious side effects associated with those trials. For instance, clinical trials of curcumin have revealed DNA fragmentation in the presence of P450 and other deleterious drug interactions. It is therefore prudent to fully understand the mechanism of action of experimental drugs before trying them on humans [191, 192]. Furthermore, although some pathways have been studied as therapeutic targets, the actual mechanisms underlying these pathways are yet to be understood. For example, inhibition of TLR4 signaling has been identified to be a preventive or treatment mechanism for ICH. Inhibition of TLR4 has been found to be either by deletion of TLR4 gene or by anti-TLR4 antibodies; however, there is still the need for studies into the specific antagonist for TLR4. Knowledge about the most critical ligand as well as the specificity of TLR4 signaling across similar cells is yet to be understood [193]. It is therefore not surprising that although a milestone in animal

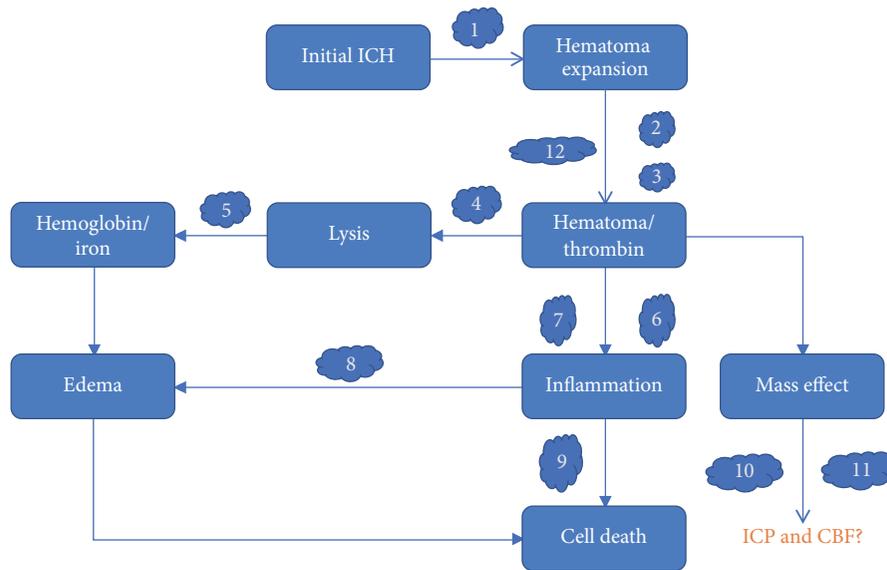


FIGURE 2: Potential therapeutic targets and treatments for ICH: 1. beta blocker; 2. rFVII; 3. desmoplasmin; 4. PPAR γ ; 5. deferoxamine; 6. celecoxib; 7. NXY-059; 8. fingolimod; 9. stem cell therapy; 10. mannitol; 11. hypertonic saline; 12. Pioglitazone.

TABLE 2: Preclinical drugs and their potential function.

Experimental drugs	Target	Function
Curcumin	Inhibit MMP-9, suppress TNF- α and iNOS	Neuroprotection, edema alleviation
Progesterone	Inhibit MMP-9, restore (BDNF)mRNA, NaK-ATPase mRNA, MAP, and ChAT	Neuroprotection, decrease glial cell and edema formation
(-)-Epicatechin	Upregulate Nrf2	Alleviates oxidative stress,
Prostaglandin E2	Downregulate HMGB1, interleukin-1 β and Src kinase expression	Prevent edema formation, neuroprotection
Melatonin	Downregulate MMP-2 and MMP-9	Neuroprotection and edema alleviation
Imatinib	Downregulate PDGFR- β	Neuroprotection
Sparstolonin	Inhibit TLR2/TLR4 and NF- κ B	Neuroprotection
Dexamethasone	Increase Bcl-2/Bax, downregulate ICAM-1 and MMP-9	Edema alleviation
Aprotinin	Inhibit plasma kallikrein	Prevents rebleeding and edema formation

studies has been achieved, we are yet to see translation into clinical trials. Experimental errors and inconsistencies in result across different laboratories also make it a challenge to translate studies of experimental drugs into clinical use. This might be due to lack of transparency and accurate data collection of experimental data. These inconsistencies will therefore mean that although one experimental result showed positive results, other laboratories might fail to replicate these results, making clinical applications impossible. We therefore are of the view that extra studies are needed in small and large animal to unravel the mysteries behind ICH disease process as well as improve limitations of preclinical studies into clinical applications.

6. Conclusion

In this review, we looked at some conservative treatment options of ICH from preclinical studies as seen in Figure 1. We also focused on some translational studies and trials that are ongoing and those completed. Although some novel treatment therapies have been developed to treat ICH, there still remains a lot to be discovered. There are still new drugs that have been experimented to be efficient in small animal models but are yet to be tried in large animals (Table 2) and then the clinics. There is the need for in-depth studies into these new drugs. Furthermore, limitations associated with translational studies of these potential therapeutic modalities

TABLE 3: Previous and current clinical trials.

Agent	Name of study	Result	Trial Number	Target
Mannitol		No significance		Edema [16]
rFVIIa	FAST	Ongoing	NCT00127283	Hematoma regrowth [17]
Fingolimod		Phase 2	NCT02002390	Edema [18]
NXY059	CHANT	No significance		Free radical [19]
Deferoxamine	DFO-ICH	Ongoing	NCT02175225 NCT02367248	Iron chelation & perihematomal edema
Pioglitazone	SHRINC	Phase 3	NCT00827892	PPAR γ -agonist [20]
Celecoxib	ACE-ICH	Ongoing		cyclooxygenase-2
Hypertension	CHANT	Ongoing		Hematoma regrowth & BP control [20]
	INTERACT	Completed		Hematoma regrowth & BP control [21]
	ADAPT	Completed		Hematoma regrowth & BP control [22]
	ATTACH	No significance		Hematoma regrowth & BP control [23]
Stem cell		Ongoing	NCT02065778 NCT02245698 NCT01832428 NCT01714167	Many targets [24]
Electroacupuncture		Ongoing		Many targets

should be curtailed, to enrich the treatment options of this complicated condition.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

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Review Article

White Matter Injury and Recovery after Hypertensive Intracerebral Hemorrhage

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Hypertensive intracerebral hemorrhage (ICH) could very probably trigger white matter injury in patients. Through the continuous study of white matter injury after hypertensive ICH, we achieve a more profound understanding of the pathophysiological mechanism of its occurrence and development. At the same time, we found a series of drugs and treatment methods for the white matter repair. In the current reality, the research paradigm of white matter injury after hypertensive ICH is relatively obsolete or incomplete, and there are still lots of deficiencies in the research. In the face of the profound changes of stroke research perspective, we believe that the combination of the lenticulostriate artery, nerve nuclei of the hypothalamus-thalamus-basal ganglia, and the white matter fibers located within the capsula interna will be beneficial to the research of white matter injury and repair. This paper has classified and analyzed the study of white matter injury and repair after hypertensive ICH and also rethought the shortcomings of the current research. We hope that it could help researchers further explore and study white matter injury and repair after hypertensive ICH.

1. Introduction

Hypertension is one of the three leading risk factors for global disease burden [1], which is known to be a basic risk factor for stroke. In 2010, approximately 16.9 million incident strokes occurred, which added up to a pool of 33 million stroke survivors worldwide [2, 3]. Hemorrhagic stroke accounted for about 31.52% of all strokes, and the most common origin is hypertension (30–60%) [2]. The most frequent occurrence location of hypertensive ICH is around the basal ganglia and thalamus, which could easily lead to death or disability.

White matter fibers, especially located within the capsula interna, are one of the most vulnerable tissues in hypertensive ICH. Quantities of pathogenic factors, which are generated by hypertensive ICH, could impact the structure and function of white matter fibers. A study found that white matter injury might reflect the vulnerability of individual brains to pathologic insults and suggested that it should be considered when assessing immediate, early, and long-term outcomes after ICH [4]. At present, white matter injury and repair after hypertensive ICH have drawn more and more attention

from researchers. However, the current study of white matter injury and repair after hypertensive ICH is still scanty and scattered. Besides, animal research showed that scores of therapeutic agents and methods were effective in the treatment of white matter injury after hypertensive ICH, while these drugs and treatment methods are rarely used in clinical practice. For one thing, it is the deficiency of animal model; for another, we need to rethink our research strategy on white matter injury and repair after hypertensive ICH.

There is no animal model able to completely simulate the natural process of human white matter injury after hypertensive ICH at present. Each animal model has its drawbacks, but each one could be used to meditate on certain pathophysiological aspects of the white matter injury after hypertensive ICH. On the other hand, as it is known, the white matter might be simultaneously or sequentially damaged by pathogenic factors which are evoked by hypertensive ICH. Owing to that, the work for a single pathogenic factor does not have an edge on the effective treatment of white matter injury and promoting its recovery. And these pathogenic factors could also damage the other brain tissues

around the damaged white matter, as blood vessels and nerve nuclei [5, 6]. In terms of functioning, brain structures influence and interact with each other. They are operating as a whole. Unfortunately, we inspected them separately. In an effort to overcome these shortcomings, we need a new research paradigm which should consist of a bleeding artery, the damaged white matter and its peripheral nerve nuclei, and so forth.

In this review, we initially depict the white matter injury after hypertensive ICH in detail, followed by a thorough elaboration of the current strategies for control of the white matter injury. Thereafter, we comment on past and current relevant studies and finally propose the lenticulostriate artery-neural complex (LNC) as a new research paradigm.

2. White Matter Injury after Hypertensive ICH

2.1. The General Epidemiology of White Matter Injury after Hypertensive ICH. White matter injury, which is caused by hypertensive ICH, occurs rather rapidly. For example, hypertensive ICH could immediately cause hemorrhagic hypotension, which reduces the systemic arterial blood pressure by 35–45% [7]. During this period, the lowest cerebral blood flows were in the white matter [7], while blood flows to all brain regions would increase by 31–42% over steady-state values after 5 minutes of hemorrhagic hypotension [7]. Dramatic changes of cerebral circulation within such a short period of time could damage the white matter and then affect the level of consciousness and other cerebral functions [8]. Thus, aggressive and early medical intervention is necessary to reduce white matter injury after hypertensive ICH.

The main location of white matter injury and that of hypertensive ICH are closely related. Different cerebral structures (such as blood vessels, white matter, and nerve nuclei) exhibited differential insensitivity to the effects of hypertension. Therefore, the occurrence of hypertensive ICH has obvious anatomic sites preference. A study in China found that hypertension was seen in 79.1% of basal ganglia and 68.2% of thalamic ICH patients, but in only a minority of cerebellar (22.2%) and lobar (20.2%) ICH cases [9]. Moreover, the majority of single hemorrhages were found in deep (subcortical) sites, including the basal ganglia (34.2%), thalamus (8.3%), cerebellum (6.8%), ventricles (1.5%), and brainstem (1.1%) [9]. Hence, around the basal ganglia and the thalamus are the main sites of white matter injury after hypertensive ICH. Research on white matter injury after hypertensive ICH should focus on these sites in the future.

The damaged degree of white matter is determined by the spatial and temporal relationship with the hypertensive ICH. A study pointed out that, one day after ICH, massive dMBP⁺ (degraded myelin basic protein) white matter tracts were seen in the core and at the edge of the hematoma, whose morphology was relatively normal; by 3 days, dMBP staining was lighter and the white matter tracts were more fragmented and larger; at a later time after ICH (28 days), dMBP was not detected in the ipsilateral striatum in laboratory animals, even inside or at the edge of the lesion [10]. But the area with dMBP increased between 6 hours and 1 day in the

ICH model but did not exhibit further changes by 3 days [10]. Interestingly, the authors did not detect dMBP in the parenchyma outside the hematoma at any of the time points [10]. But other evidence suggested that the existence of early moderate ischemia could cause white matter lesion, in the parenchyma outside the hematoma. For instance, multimodal monitoring demonstrated hematoma volume-dependent changes of tissues oxygenation, blood flow, and ischemic microdialysis markers in the gray-white matter junction for 12 hours of monitoring [11]. This reminds us of the issue of whether a slight white matter lesion existed in the parenchyma outside the hematoma, which requires further research in the future work.

2.2. Pathogenic Factors of White Matter Injury after Hypertensive ICH. Besides the direct cell death (including but not limited to apoptosis, necrosis, autophagy, and recently necroptosis) in oligodendrocytes, myelin, or axon, a good many pathogenic factors (Figure 1) could be involved in white matter injury after hypertensive ICH, such as the hemorrhagic brain edema, the mass effect of the hematoma, the hemodynamic change of the cerebral circulation, and the inflammatory response induced by the blood components and metabolites. The white matter is simultaneously or sequentially damaged by these pathogenic factors after hypertensive ICH, while other damaged brain tissues around the white matter could conversely generate cell death of white matter components.

The hemorrhagic brain edema after hypertensive ICH could quickly reduce the metabolic rate and then cause injury of the white matter. Animal experiments showed that, compared with the control group, glycogen and glucose concentrations, respectively, increased twofold to fivefold during a period of 8 hours after infusion; phosphocreatine levels increased severalfold by 5 hours; and lactate was obviously increased (approximately 20 mMol/g) at 1, 3, 5, or 8 hours after infusion in markedly edematous white matter [12]. In addition, the hemorrhagic brain edema after hypertensive ICH occurs promptly and lasts longer. Previous studies in pig models indicated that edematous white matter areas were present directly around the hematoma at 1 hour after ICH [13]. This region had a greater than 10% increase in water content (>85%) compared with the contralateral white matter (73%), and this increased water content persisted through 8 hours [13]. And a clinical study found that the patient's edema volume at the second week went up in comparison with the first week after hypertensive ICH; and the edema volume in the fourth week only returned to the same level as in the first week [14]. Sudden and prolonged brain edema after hypertensive ICH are mainly driven by blood vessel disruption and serum proteins and plasma proteins (such as albumin and IgG) accumulation in the white matter around the hematoma [13, 15–17]. Interestingly enough, hypertensive ICH could elevate the content of cerebral interstitial serum proteins through cerebral vessels about 3-fold compared with normotensive ICH in laboratory animals [18]. And later animal experiments found that serum proteins derived from blood clot formation in normotensive ICH [16]. This is an indication that white matter injury, which is caused by

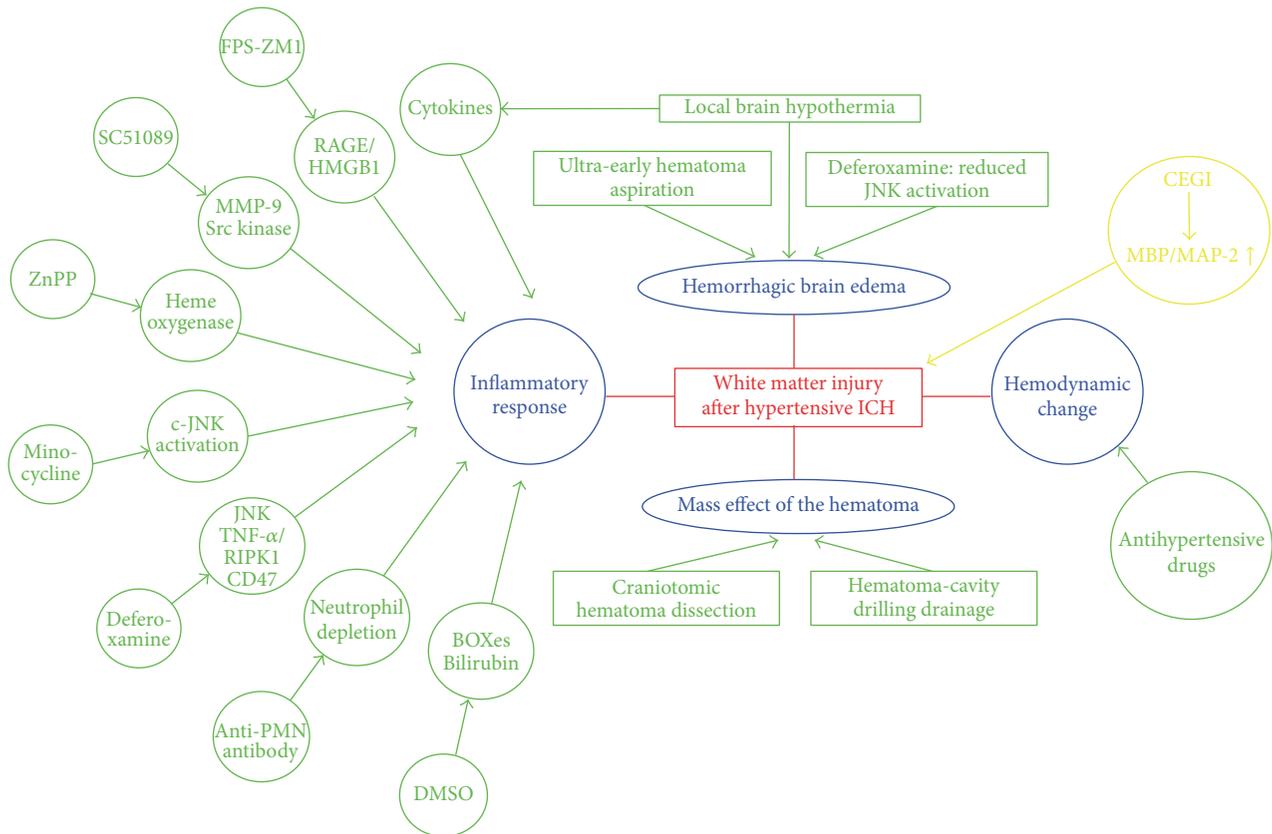


FIGURE 1: Schematics for the pathogenic factors of white matter injury after ICH.

edema after hypertensive ICH, has its specialties and that distinctions in studies should be made in the future.

Another major etiological factor after hypertensive ICH is the mass effect of the hematoma, which could reduce CBF and then cause ischemic damage of the white matter. The mechanical microballoon model is applied frequently to simulate the effect. Previous research revealed that experimental animals exhibited significant ischemic damage and reduced CBF persisted for 4 hours after transient inflation of a microballoon in the caudate nucleus [19]. Clinical research also showed us that subcortical white matter might be damaged by the mass effect when the volume of the hematoma exceeds around 25 mL after hypertensive ICH [20]. And our recent findings suggested that the mass effect of the hematoma could cause direct pathologic damage to the white matter by the mechanical microballoon model in rats [21]. Although surgical efficacy of removal of intracerebral hematoma has always been controversial [22, 23], it is still valuable for us to research the relationship between the mass effect of hematoma and the white matter injury.

The hemodynamic change of the cerebral circulation is also one of the important causes for white matter injury after hypertensive ICH. Hemodynamic parameters experienced a dramatic shift in a short time after hypertensive ICH. As is stated above, hypertensive ICH could immediately cause hemorrhagic hypotension, which reduced the systemic arterial blood pressure by 35–45% in experimental animals

[7]. Yet, a large number of clinical trials discovered that the mean systolic blood pressure within 3 hours of hypertensive ICH was substantially higher than pre-morbid levels (mean increase of 40.7 mmHg, $p < 0.0001$) [24]. These changes will inevitably affect the white matter blood flow. Animal experiments have shown that the lowest cerebral blood flows were in the white matter during the hemorrhagic hypotension period, but the blood flows of the white matter increased by 31–42% over steady-state values after 5 minutes [7], which is a serious injury to the white matter. Therefore, sustaining steady hemodynamics of cerebral circulation might be contributed to alleviate the white matter injury after hypertensive ICH.

After hypertensive ICH, the inflammatory response, which is induced by the blood components and metabolites, was severely destructive to the white matter. The components of plasma (such as thrombin, complement, glutamate, and carbonic anhydrase 1) and released substances from the hematoma (such as hemoglobin and iron) could cause inflammation and damage through promoting the activation of resident microglia and the production of inflammatory mediators by influx of leukocytes into the brain [25, 26]. For instance, animal research found that plasma protein led to rapid white matter injury through inducing a cascade of acute inflammatory events including oxidative stress, proinflammatory cytokine gene expression, and DNA damage within 24 h after ICH [27]. In addition, blood breakdown products

could also cause white matter injury in a delayed manner, for a study found that bilirubin and bilirubin oxidation products could affect the structural integrity and function of white matter tracts of the corpus callosum at 7 days after ICH [28]. Accordingly, for the white matter injury after hypertensive ICH, we should pay attention to the delayed inflammatory response as well as acute inflammatory response in the future.

To sum up, the white matter injury after hypertensive ICH was caused by a variety of pathogenic factors. It is very difficult for single therapeutic strategies to prevent and treat white matter injury after hypertensive ICH. So, multiple therapeutic interventions and multipotential drugs should be taken seriously in the future work.

2.3. Pathological Changes of Cerebral White Matter after Hypertensive ICH. The pathological changes of white matter after hypertensive ICH were blatantly obvious. Recent animal experiments showed that the white matter was progressively lost in the perihematoma from day 1 to day 28 after ICH [29]. Myelin sheaths played an important role in maintaining the morphology and function of the white matter. Hypertensive ICH could lead to demyelination and downregulation of MBP expression in the white matter. Morphologic changes of myelin sheaths included swelling and damage at first, followed by demyelination and lastly oligodendrocyte apoptosis after ICH [30]. Accompanying this, a large number of pathological molecules were highly expressed or overactivated, such as TNF- α [31], RIPK1 [32], receptor for advanced glycation end-products (RAGE), HMGB1 [33], CD47 [34], SC1 [35], β -APP [36], and JNK signaling pathway [37]. These pathological molecules had aggravated the damage of myelin sheaths and then affected the morphology and function of the white matter. Meanwhile, it should be noted that the self-repairing function of brain tissue played an active role, as the densities of immature oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes in the perihematoma increased dramatically over the first week after ICH in rats [38]. Therefore, it may be a better strategy to improve the self-repairing function of brain tissue in the treatment of white matter injury after hypertensive ICH.

2.4. Consequence of White Matter Injury on Patients after Hypertensive ICH. White matter injury will seriously affect the prognosis of hypertensive ICH patients. A study found that severe white matter injury is a prognostic factor for poor activities of daily living at discharge in elderly patients with stroke [39]; and severe leukoaraiosis is also independently associated with the long-term mortality in survivors after ICH [40]. The clinical manifestation of ICH patients is strongly connected with the location and the degree of white matter injury. As already mentioned above, around the basal ganglia and thalamus are the main sites of white matter injury after hypertensive ICH. White matter injury in this region could bring about persistent aphasia [20], buccofacial apraxia [41], ideational apraxia [42], dysgraphia [43], and so forth. But not all white matter injuries could contribute to these clinical features, just as the volume of hematoma exceeding around 25 mL is the precondition for the occurrence of white matter damage [20]. To conclude, although we found

that white matter injury is closely related to some clinical features, there are relatively few studies in this field and the pathogenic mechanism is unclear. It is crucial to further explore the relationship between white matter injury and clinical manifestations.

2.5. Detection Methods for the White Matter Injury after Hypertensive ICH. The accurate detection of white matter injury helps in the prevention and treatment of hypertensive ICH (Table 1). At present, the most extensive detection technique used for white matter injury is magnetic resonance imaging (MRI). For detecting white matter injury, diffusion tensor imaging (DTI) and T1- and T2-weighted spin-echo sequences (T1WI and T2WI) could all be used as certain types of MRI [44–46]. DTI tractography was the most suitable for assessing longitudinal changes in white matter fibers' integrity and mechanical displacement [44, 47]. Pathology-affected white matter fibers in patients with ICH could be selectively visualized by using structural neuroimaging and DTI volumes [44]. Combined usage of T1WI and T2WI in patients with hypertensive ICH could discover white matter injury, such as widespread white matter edema [48], hemorrhagic lacunes [45], and white matter hyperintensities [49]. However, T1WI and T2WI could not intuitively understand the damage of white matter fibers compared with DTI. As a result, if accurate study of the white matter injury with MRI is required, DTI should be the top priority.

Computed tomographic (CT) scan is another technique for detecting white matter injury after hypertensive ICH. Being able to quickly identify and differentiate hemorrhagic stroke from ischemic stroke is of extremely important significance for the clinic. The main manifestation of white matter damage on CT is reduced white matter density (leukoaraiosis) [50, 51]. Significant leukoaraiosis has been found in about 38% of cerebral apoplexy patients [50]. In order to distinguish the degree of white matter damage more accurately, leukoaraiosis was scored on the baseline of CT scan as described by van Swieten et al., with an overall score from 0 to 4 [40, 52]. Although efforts had been made, CT is still unable to catch up with MRI in the detection of white matter injury. Therefore, the relevant research reports are few.

Pathological examination is the principal method for diagnosis. The pathological changes of white matter after hypertensive ICH could be quickly and accurately found by immunohistochemical staining, western blotting, southern blotting, PCR, and so forth [29, 30, 33]. The pathological changes of white matter after hypertensive ICH were involved not only in morphology, but also in function. Only pathological examination could perfectly solve these problems. Besides, pathological examination could also be used to assess the self-repairing function of the white matter after hypertensive ICH; namely, the densities of immature OPCs and mature oligodendrocytes in the perihematoma were determined by pathological examination [38]. Even though pathological examination has many advantages, as an invasive examination, it could not possibly be widely carried out in patients with hypertensive ICH.

In addition, there are indirect ways to detect white matter injury after hypertensive ICH; for example, white matter

TABLE 1: The detection methods of white matter injury.

Detection method	Advantage	Disadvantage	Detection mode
Diffusion tensor magnetic resonance imaging	(1) Assessing longitudinal change in white matter fibers (2) Selectively visualizing the white matter fibers (3) Early detection of the white matter injury	(1) Expensive (2) Requires higher hardware and software	Direct
T1- and T2-weighted spin-echo sequences magnetic resonance imaging	(1) Wider application (2) Roughly assessing the damage situation of white matter	(1) Expensive (2) Unable to early detect the white matter injury (3) Unable to clearly visualize the white matter fibers	Direct
Computed tomographic scan	(1) Inexpensive (2) Wider application (3) Roughly assessing the damage situation of white matter	(1) Unable to early detect the white matter injury (2) Unable to clearly visualize the white matter fibers	Direct
Laser Doppler	(1) Inexpensive (2) Quickly and accurately detecting the blood flow changes of white matter	(1) Unable to directly detect the white matter injury (2) Invasive examination	Indirect
Pathological examination	(1) Cheap (2) Has a variety of detection means (3) Quickly and accurately finding the change in white matter fibers	Invasive examination	Direct
Radioactive microsphere technique	Quickly and accurately detecting the blood flow changes of white matter	More difficult to apply in clinic	Indirect
Poststroke activities of daily living (ADL)	(1) Easy to use and cheap (2) Quickly detecting white matter injury	Only indirectly and roughly judged white matter injury	Indirect
Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE)	(1) Easy to use and cheap (2) Quickly detecting white matter injury	Only indirectly and roughly judged white matter injury	Indirect
Modified Telephone Interview for Cognitive Status test	(1) Easy to use and cheap (2) Quickly detecting white matter injury	Only indirectly and roughly judged white matter injury	Indirect

blood flow changes after hypertensive ICH could be detected using laser Doppler and the radioactive microsphere technique [7, 53]. Moreover, poststroke activities of daily living (ADL), Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), and the Modified Telephone Interview for Cognitive Status test could all be used to indirectly determine the degree of white matter injury of hypertensive ICH patients [39, 54, 55].

In summary, none of the methods above are flawless. It is of vital importance to find noninvasive and more accurate detection methods for further study of white matter injury after hypertensive ICH.

3. Current Strategies for White Matter Injury after Hypertensive ICH

3.1. Therapeutic Strategies for the Pathogenic Factors. As stated above, of the various pathogenic factors that resulted in white matter injury after hypertensive ICH, the mass effect of hematoma was the most vital one. Surgical removal of

the hematoma could relieve oppression of the surrounding brain tissue, lower intracranial pressure, relieve and prevent cerebral hernia, improve cerebral blood flow, reduce sequela, and so forth. Hence, this is a good way. There are two main surgical methods for the removal of hematoma: one is the craniotomic hematoma dissection and the other is the hematoma-cavity drilling drainage [56]. Yet, there is no study on whether the surgical removal of the hematoma could reduce the damage of white matter at present. On the other hand, the hemorrhagic brain edema is another pathogenic factor causing white matter injury after hypertensive ICH. Blood clot formation could cause the occurrence of brain edema after ICH [16]. It was reported that ultra-early hematoma aspiration after fibrinolysis with t-PA in a porcine model of ICH notably reduced perihematomal edema and protected the blood-brain barrier [57]. This suggests that surgical removal of the hematoma may help reduce white matter injury through different mechanisms, which is worth of intensive study in the future.

Using drugs to regulate the expression and the related pathogenesis of pathogenic molecules is another strategy to

reduce the damage of white matter after hypertensive ICH. Some drugs had been shown to reduce the damage of white matter in animal models of ICH, just as FPS-ZMI (RAGE-specific antagonist) could relieve the damage of white matter via antagonism of ligand/receptor interaction [33]; SC51089 (EPIR antagonist) could decrease Src kinase phosphorylation and MMP-9 activity and then relieve the damage of the white matter [58]. Zinc protoporphyrin (ZnPP), as a heme oxygenase inhibitor, could also reduce white matter injury [29]; and minocycline was able to suppress Fe-induced white matter injury and c-JNK activation after hypertensive ICH in rats [36]. As a crucial component of the white matter, axon damage after ICH is relatively common. A study found that dimethyl sulfoxide or its structurally related derivatives, which could effectively attenuate the toxic effect of bilirubin and its oxidation products, might have a potential therapeutic value in antagonizing axonal damage after ICH [28].

Deferoxamine is a medicine which captures our attention. Previous animal research found that deferoxamine could remarkably reduce white matter injury after ICH via multiple mechanisms, including inhibiting white matter edema [37], reducing ICH-induced JNK activation [37], lowering TNF- α and RIPK1 levels [32], and upregulating the expression of CD47 [34]. In view of the good performance of deferoxamine in the animal experiment, a clinical trial was carried out and went on well. Currently, third-phase clinical trials of deferoxamine are underway. Hopefully, it would be widely used in clinical treatment in the foreseeable future.

In addition, two other interesting therapeutic methods are noticeable for white matter injury. One is the delayed profound local brain hypothermia. This study found that the delayed profound local brain hypothermia was able to protect white matter tracts through strikingly reduced inflammatory cytokines production and vasogenic edema development in a porcine model of ICH [59]. The other is neutrophil depletion. Circulating blood neutrophils were depleted with an anti-PMN antibody before inducing ICH in rats. After experiments, detailed spatial analysis showed that neutrophils depletion reduced infiltration of activated microglia/macrophages in the perihematoma white matter tracts and decreased myelin fragmentation and axon damage [17, 60].

3.2. Therapeutic Strategies for White Matter Restoration after Hypertensive ICH. Due to less attention on the restoration of white matter injury after hypertensive ICH in the past, there is not much evidence for white matter restoration after hypertensive ICH. Inspired by a few recent studies, endogenous or exogenous stem cell therapy [61, 62], as well as new drugs towards the pathogenic factors summarized in Section 2.2, may be the promising strategy to promote the repair of damaged white matter injury after hypertensive ICH. Joseph et al. found that endogenous oligodendrocyte precursors could proliferate and differentiate in the perihematoma region and had the potential to remyelinate axon tracts after ICH [38]. And another animal experiment also found that cattle encephalon glycoside and igitonin (CEGI) treatment could effectively upregulate MBP/MAP-2 expression, ameliorate white matter fibers damage, and alleviate the neurobehavioral

dysfunction after ICH [63]. Minocycline and deferoxamine were also demonstrated to be protective towards white matter injury after ICH, possible via iron clearance [32, 36, 37, 64]. And ZnPP reduced white matter injury via reducing heme degradation products after ICH [29]. Our previous study indicated that the receptor for advanced glycation end-products (RAGE) antagonist alleviated axon injury in the white matter after ICH [11]. For further translational studies, more attention is needed to explore the repair mechanism and related drugs of the white matter injury after hypertensive ICH in the future.

As demonstrated above, progress has been made on the treatment of white matter injury after hypertensive ICH. On the other hand, the relevant research in this field is still scanty and the targeted treatment is also limited. The key to solve the problem is to explore in depth the white matter injury after hypertensive ICH.

4. Animal Model for White Matter Injury after Hypertensive ICH

Through reviewing the relevant studies, there are three main methods to construct the white matter injury model: microballoon infusion [19], collagenase injection [48], and autologous blood injection [13]. The advantage of the microballoon infusion model is that it could effectively simulate the mass effect of the hematoma. Thereby, it has contributed to the evaluation of the mass effect of hematoma on the white matter. The disadvantage is that it fails to address the potential effects of the blood components, metabolites, and subsequent substances released by the clot formation [65]. The advantage of collagenase injection model is that it could accurately simulate ICH in humans and avoid technical difficulties in handling blood [66]. Furthermore, it also simulates the hematoma expansion of continuous bleeding that occurs naturally in ICH patients [67, 68]. For this reason, it is more accurate to simulate the occurrence and development of white matter injury after ICH, while this model could produce excessive inflammatory response and other non-hemorrhage-related effects which could affect the assessment severity of white matter injury [25, 66]. The autologous blood injection could imitate the effects of an intracerebral hematoma in the brain while avoiding the disadvantage of the collagenase injection model, which is helpful for us to study the damage of the blood components and metabolites to white matter after ICH. Nevertheless, it does not accurately simulate the occurrence of hypertensive ICH as the cerebral vasculature was not disrupted [19].

Laboratory animals used for white matter injury studies after ICH include canines [69], pigs [11], cats [70], rabbits [71], rats [36], and mice [58]. Both rats and pigs were widely used in white matter injury research. Rats were the most widely used in the research of white matter injury thanks to their competitive prices, easy accessibility, and easy anesthetic operation. At the same time, the relatively smaller brain size makes them difficult to simulate well-developed white matter in human brain. Known for their large, gyrated brain and well-developed white matter, pigs are an ideal animal for

the study of white matter injury after hypertensive ICH, for example, early perihematomal edema. And the study found that the large hematoma volume in pigs after ICH was more limited to the target area compared to other animal species [13, 72]. Pigs also have their challenges, especially relatively higher purchase price and a larger volume.

In summary, there is no ideal animal model to simulate the natural process of human white matter injury after hypertensive ICH at present. But each model could be used to study certain pathophysiological aspects of the white matter injury after hypertensive ICH. That is to say, on the one hand, in the future, we need to choose methods of structuring animal models according to different research purposes; on the other hand, continuous exploration and establishment are demanded as for the new animal models, which is more consistent with the natural process of human hypertensive ICH. And the new animal models should be easily induced, relatively cheap, convenient, and effective for the studies of the pathophysiological mechanisms of white matter injury and repair.

5. A New Research Paradigm of White Matter Injury after Hypertensive ICH

5.1. Hypertension for the Susceptibility and Pathological Changes. It is known that hypertension is the most essential pathogenic factor of hypertensive ICH. Previous studies have found that chronic hypertension could induce hypertrophy of intracerebral arterioles by increasing the expression of the vascular ECM, like fibronectin, laminin, and collagen IV [73]. This is an indication that chronic hypertension could affect blood supply of the white matter before onset of ICH, which was confirmed by recent research. The study found that hypertension disrupts the structure and function of cerebral blood vessels, which leads to ischemic damage of white matter regions critical for cognitive function [74]. What is more, another cerebral finding in chronic hypertension was hypertensive encephalopathy, in which breakdown of the blood-brain barrier to serum proteins occurred in multifocal areas of the cortex and basal ganglia [75]. Mentioned earlier, the blood-brain barrier damage could lead to brain edema and then cause white matter injury. This prompted us to think about whether the treatment of chronic hypertension is helpful to reduce white matter injury after hypertensive ICH [76].

In addition to that, antihypertensive therapy after hypertensive ICH might also have a positive effect on relieving white matter injury. A study found that systolic blood pressure was substantially raised compared with usual pre-morbid levels after ICH [24]. However, previous researches indicated that lower blood pressure is not always good for ICH patients. In fact, after hypertensive ICH, the benefits of early treatment to reduce systolic blood pressure to 140 mmHg might be enhanced by smooth and sustained control and particularly by avoiding peaks in systolic blood pressure [77]. And as interpreted above, the severe hemodynamic change of the cerebral circulation could cause damage to the white matter after hypertensive ICH. Apparently, there exists a

correlation between hypertension and white matter injury after hypertensive ICH.

To conclude, hypertension could induce white matter injury. Currently, research considered that ischemic damage was the main pathogenic mechanism of white matter injury caused by hypertension. However, the relationship between hypertension and white matter injury has not been further investigated after hypertensive ICH. The pathogenic mechanism of white matter injury caused by hypertension also needs in-depth exploration and discussion. Moreover, whether the treatment of chronic hypertension is useful to reduce the white matter damage after hypertensive ICH is an intriguing topic. It is enchanting that plenty of inspiring work is waiting to be discovered about hypertension and white matter injury.

5.2. Lenticulostriate Artery-Neural Complex. As indicated in previous sections, a number of pathogenic factors caused white matter injury after hypertensive ICH. Clearly, these pathogenic factors are interrelated. This reminds us that the white matter is simultaneously or sequentially damaged by different pathogenic factors after hypertensive ICH, not to mention that hypertensive ICH could also damage other brain tissues around white matter injury, like blood vessels and nerve nuclei. Evidently, these damaged brain tissues could conversely generate white matter injury. Far from it, through the review of the previous studies on white matter injury, it is the damage of the single pathogenic factor that these therapeutic studies focus on. Animal experiments mainly focus on the inflammatory response which is induced by the blood components and metabolites, while clinical trials are mainly emphasized in the mass effect of hematoma, and what these studies have in common is that they all did not pay attention to the effects of the damaged surrounding brain tissues on the white matter. Out of question, previous studies have improved our understanding of white matter injury after hypertensive ICH. And based on these studies, we have identified dozens of therapeutic agents and methods for the treatment of white matter injury after hypertensive ICH. And yet we should also take notice that many drugs and methods are rarely used in clinical practice. Therefore, we need to rethink our research strategy on white matter injury after hypertensive ICH.

In the past 20 years, there has been a dramatic change in the research paradigm of stroke pathophysiology. For example, preliminary studies found that neuroprotection alone for ischemic stroke could not yield a benefit. Thus, the neuronal-astrocytic-vascular tripartite functional unit was initially proposed in 1996 [78]. Cohen and others believed that dysfunctions in these neurovascular interactions might result in perfusion deficits and might be involved in specific pathological conditions [78]. This concept was revised and named the neurovascular unit at the first Stroke Progress Review Group meeting in 2001 [79]. The neurovascular unit emphasized the complexity of interactions between all perivascular cell types [80]; and it integrated neural and vascular cell types to help explain the failure of neuroprotective strategies for ischemic stroke [81]. But the neurovascular

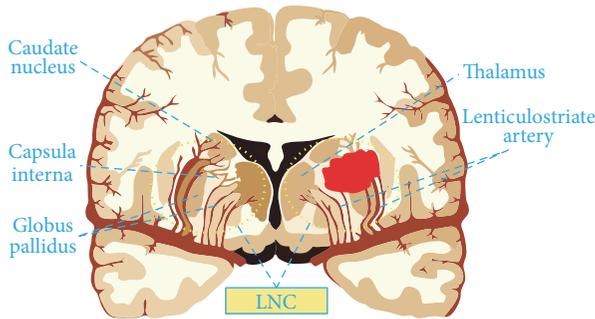


FIGURE 2: Illustrations for the lenticulostriate artery-neural complex.

unit model focuses largely on the areas immediately surrounding capillaries, where neural and vascular cells interact and influence each other, and excludes downstream venous vasculature, upstream arterioles, and smaller arteries [80–82]. In 2012, Zhang and others proposed the vascular neural network as a new paradigm that combines the original concept of the neurovascular unit with emerging understanding of the key roles of arterial smooth muscle cells, endothelial cells, and perivascular nerves in cerebrovascular physiology and pathology [81]. This paradigm prominently promoted the study of stroke. In view of it, the research paradigm, which focuses on pathogenic factor alone, could not accurately reveal the pathophysiology of white matter injury after hypertensive ICH. In consequence, based on the anatomic structures of the most common bleeding site, we propose the research paradigm of the lenticulostriate artery-neural complex (LNC) (Figure 2), which is highly expected to contribute to the study of white matter injury after hypertensive ICH.

Lenticulostriate artery-neural complex is composed of the lenticulostriate artery and its hemodynamic system, nerve nuclei of the hypothalamus-thalamus-basal ganglia system, and the white matter fibers were located within the capsula interna. With this specific and representative structure as research paradigm, the LNC has the following superiorities. To start with, it is instrumental to avoid the limitations of previous studies on white matter injury after hypertensive ICH. And it will make us pay more attention to the early warning of white matter injury, the role of biomechanics in the white matter injury, the overall protection of white matter and surrounding nerve nuclei after hypertensive ICH, and so forth. Secondly, not only could it explain why the predilection site of hypertensive ICH is the lenticulostriate artery, but also it could be used to clarify the role of the hemodynamic parameters changes and the stress boundary conditions of perivascular area in the white matter injury caused by hypertension. What is more, it is beneficial to the coupling of hemodynamics, endothelial cells, vascular smooth muscle cells, peripheral nerve nuclei, and so on. Additionally, the occurrence and development of white matter injury and repair after hypertensive ICH were studied from the cellular and molecular levels. Last but not least, it could also be used to illustrate the pathological changes

and clinical manifestations of patients with hypertensive ICH. Just as a patient with hemiplegia, hemianopia and hemidysesthesia result from the white matter fibers injury within the internal capsule after hypertensive ICH; the mass effect of hematoma, hypothalamus injury, and brain edema formation are substantial factors leading to lethal hernia after hypertensive ICH; and long-term brain atrophy and cognitive impairment after hypertensive ICH are related to the toxicity of the blood components, metabolites, and excessive inflammation. In brief, the LNC could systematically take into account the roles and mechanisms of hemodynamics, blood vessel injury and rupture, hematoma stress injury, white matter fibers, nerve nuclei (hypothalamus, thalamus, and basal ganglia), and blood component and its metabolites; immunologic and inflammatory response in the onset and development of hypertensive ICH is of vital significance in the exploration of white matter injury and repair.

Research around the LNC is wholesome to display the mechanism of the occurrence and development of white matter injury and repair after hypertensive ICH. More importantly, it will contribute to the establishment of an early warning system, early diagnosis, and early intervention and repair strategies. Specifically, it mainly contains the following four aspects. Initially, we need to seek out the genetic and environmental risk factors and their interactions in the pathogenesis of white matter injury induced by hypertension and to establish an early warning molecular system. And then, we need to study the mechanism of hemodynamic and vascular coupling injury in the pathogenesis of hypertensive ICH and the specific imaging early warning signs in a state of intense change in blood pressure. Furthermore, we should work over the effect and mechanism of the stress of hematoma on the structure and function of the LNC, followed by providing a reliable scientific basis for the clinical intervention of white matter injury and meanwhile promoting repair. At last, it is also needed to investigate the role of excessive inflammatory response, which was a result of the toxic effects of blood, its metabolites, and the activation of the DAMPs, in the pathogenesis of the LNC lesion. Only in this way could we better understand the white matter injury after hypertensive ICH and its early warning and treatment.

On the whole, relatively fewer studies focused on white matter injury and repair after hypertensive ICH, and many issues still exist in the current research strategy. As a new research paradigm, the LNC helps us to better comprehend the pathophysiology of white matter injury and repair after hypertensive ICH.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Research Article

Increase of Soluble RAGE in Cerebrospinal Fluid following Subarachnoid Haemorrhage

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Receptors for advanced glycation end-products (RAGE) mediate the inflammatory reaction that follows aneurysmal subarachnoid haemorrhage. Soluble RAGE (sRAGE) may function as a decoy receptor. The significance of this endogenous anti-inflammatory mechanism in subarachnoid haemorrhage (SAH) remains unknown. The present study aims to analyse sRAGE levels in the cerebrospinal fluid (CSF) of SAH patients. sRAGE levels were assayed by ELISA kit in 47 CSF samples collected on post-SAH days 0–3, 5–7, and 10–14 from 27 SAH patients with acute hydrocephalus. CSF levels of sRAGE were compared with a control group and correlated with other monitored parameters. In the control group, the CSF contained only a trace amount of sRAGE. By contrast, the CSF of 20 SAH patients collected on post-SAH days 0–3 was found to contain statistically significant higher levels of sRAGE (mean concentration 3.91 pg/mL, $p < 0.001$). The most pronounced difference in CSF sRAGE levels between good and poor outcome patients was found on days 0–3 post-SAH but did not reach the significance threshold ($p = 0.234$). CSF sRAGE levels did not change significantly during hospitalisation ($p = 0.868$) and correlated poorly with treatment outcome, systemic inflammatory markers, and other monitored parameters. Our study revealed an early and constant increase of sRAGE level in the CSF of SAH patients.

1. Introduction

The management of aneurysmal subarachnoid haemorrhage (SAH) has seen no significant advance since the introduction of nimodipine and the use of Guglielmi detachable coils [1, 2]. Favourable outcomes occur in only about one-third of patients admitted in a poor neurological state, and no new drugs have been approved for use in SAH in the past two decades; hence there is an urgent need to look for new therapies [3–8]. Early brain injury (EBI) is considered a promising target for future research [9, 10]. This represents the pathophysiological events occurring during the first 72 h following SAH and strongly determines the mortality and morbidity [11]. Experimental models support a number of mechanisms for EBI including inflammation [9]. Further clinical studies need to determine which of these mechanisms

predominate. The present writers regard inflammation as a promising target for investigation. On a cellular level, inflammation is triggered by a ligand-receptor interaction. Among the most abundant multiligand are receptors for advanced glycation end-products (RAGE). RAGE has been shown to be present in neurons, glia, and microglia in the human hippocampus and cortex [12]. The concentration of many of its ligands (e.g., high mobility group box 1 protein, S100B protein) in plasma or cerebrospinal fluid (CSF) correlates with the clinical outcomes in patients with SAH [13–15]. Binding of these ligands to RAGE leads to the recruitment of multiple intracellular signalling molecules and eventually activates pathways responsible for acute and chronic inflammation [16]. There is a growing body of evidence that RAGE and its ligands are involved in the pathogenesis of other disorders including some cardiovascular conditions,

neurodegenerative processes, and autoimmune diseases [17]. The soluble isoform of RAGE (sRAGE) corresponds to the extracellular domain of RAGE lacking cytosolic and transmembrane domains. As a decoy receptor, sRAGE is able to bind the same ligands as a membrane-bound form but unable to trigger the intracellular responses. The anti-inflammatory potential was confirmed in a mouse experimental stroke model, where intravenous administration of recombinant sRAGE significantly reduced infarct size and improved functional outcome [18]. The soluble form of RAGE has also been widely recognised as a biomarker. CSF levels of sRAGE were observed to be reduced in Guillain-Barré syndrome and multiple sclerosis [19, 20]. In view of the presence of sRAGE in CSF, its significant role in ischaemic stroke, and the role of its ligands in SAH, we aim to analyse sRAGE levels in CSF of patients with SAH requiring acute treatment of hydrocephalus.

2. Materials and Methods

2.1. Study Population. This single-centre, observational, prospective study was conducted in accordance with the Declaration of Helsinki and its protocol was approved by the local bioethics committee. Between January 2015 and September 2016, twenty-seven patients met the enrollment criteria which are as follows: (1) SAH confirmed by computed tomography (CT), (2) early (<24 h) endovascular treatment, (3) acute hydrocephalus diagnosed on CT and managed with external ventricular drainage (EVD) < 48 h, and (4) informed consent (by patient or family). Patients below the age of 18 were excluded due to physiological differences in CSF content as well as distinct aSAH presentation, aneurysm morphology, and outcome [21, 22]. Also excluded were patients with central nervous system (CNS) disease and those with active systemic diseases (diabetes mellitus, rheumatoid arthritis, malignancy, cirrhosis, and renal failure). Meticulous care was taken to rule out patients with signs of EVD infection. CSF cell count was checked at least twice per patient and CSF culture was ordered at least once on post-SAH days 10–14. SAH management in our unit involves the continuous intravenous infusion of nimodipine for at least ten days, whilst avoiding hypotension by means of vasopressors. CT scan of the head was carried out at least twice in every patient: on post-SAH days 2–3 to assess procedure related injury and before discharge to assess delayed cerebral ischaemia. The control samples of CSF were obtained during anaesthesia from 20 patients with a negative history of CNS disease.

2.2. End Points. Subjects were followed until death or the completion of 3 months following SAH. The primary outcome was the functional state after 3 months, and the secondary outcome was in-hospital mortality. The functional outcome was defined using the Glasgow Outcome Scale (GOS); these were dichotomized as good (GOS 4–5) or poor (GOS 1–3) outcomes.

2.3. Sample Collection and Assays. CSF samples were collected from the EVD at three time points, on post-SAH

days 0–3, 5–7, and 10–14. The final sRAGE assays comprised twenty samples from days 0–3, sixteen from days 5–7, and eleven from days 10–14. A complete set of three samples was obtained from only five patients on account of suspected EVD infection, EVD obstruction, or early EVD removal. Each sample was centrifuged and stored at -80°C until assayed. The sRAGE assays were carried out using ELISA commercial kit RAB0007-1KT (Sigma Aldrich, St. Louis, USA). The minimum detectable level of sRAGE was 2.06 pg/mL, and linearity was conserved between 2.06 pg/mL and 1500 pg/mL. Haemoglobin (Hgb) level, C-reactive protein (CRP) level, and white blood cell (WBC) count and fibrinogen level were assessed daily by automatic analysers XT 2000i (Sysmex, Japan), Cobas 6000 (Roche Diagnostic, USA), and ACL TOP 500 (Instrumentation Laboratory, Italy).

2.4. Statistical Analysis. In the tables, values for numerical data have been expressed as mean and standard deviations; for ordinal numerical data, they are expressed as median and interquartile range and for categorical data as counts and percentages. In the figures, all data are presented as mean and standard deviations. The normality of data distribution was assessed using the Shapiro-Wilk test. The correlations were assessed by Spearman's test, and correlation coefficient (cc) > 0.6 (cc < -0.6) was considered significant. A value of p < 0.05 was considered statistically significant when comparing. Data were analysed using Statistica 10 (Statsoft, Inc., Tulsa, OK, USA).

3. Results

The control group consisted of twenty-five patients free of CNS disease. Detectable levels of sRAGE were found in only two members of this group (CSF sRAGE of 2.1 and 1.87 pg/mL). The details of the study group are presented in Table 1. CSF collected on days 0–3 following aneurysmal rupture in twenty of these patients contained statistically significant higher levels of sRAGE (p < 0.001) (Figure 1). No sRAGE was found in four of these twenty. Mean concentration varied significantly (0–15.22 pg/mL) but failed to differentiate good and poor outcome. The most pronounced difference between good and poor outcome was found at this stage but did not achieve statistical significance (p = 0.234) (Figure 2). The p values for days 5–7 and 10–14 were 0.291 and 0.490, respectively. Furthermore, CSF sRAGE levels did not change significantly during hospitalisation (p = 0.868) (Figure 3). Spearman's test revealed that the strongest correlation with outcome (measured by GOS at 3 months) were the admission grades Hunt and Hess (HH) (cc = -0.656), Glasgow Coma Scale (GCS) (cc = 0.688), and World Federation of Neurosurgical Societies (WFNS) (cc = -0.741), together with the fibrinogen level on days 10–14 post-SAH (cc = -0.626). sRAGE levels, haemoglobin, and blood inflammatory markers (CRP, WBC) showed poor correlation with treatment outcome (Table 2). The sRAGE levels of patients scoring 5 on the WFNS scale at days 0–3 showed a stronger correlation (cc = 0.485) with treatment outcome than those scoring 5 on the HH scale (cc = 0.176).

TABLE 1: Study group patients' characteristic.

Male	15 (56%)		
Age (years)	58.07 ± 15.8		
Aneurysm location			
Middle cerebral artery	7 (26%)		
Anterior communicating artery	7 (26%)		
Anterior cerebral artery	4 (15%)		
Basilar artery	4 (15%)		
Internal carotid artery	3 (11%)		
Posterior cerebral artery	2 (7%)		
Aneurysmal size (mm)	5.08 ± 1.8		
Cerebral infarction due to DCI on CT	20 (74%)		
Intracerebral haemorrhage on CT	14 (52%)		
Intraventricular blood on CT	26 (96%)		
Fisher CT score	4 (4-4)		
Modified Fisher CT score	4 (2-4)		
WFNS score on admission	5 (3-5)		
HH score on admission	4 (4-5)		
GCS on admission	5 (4-10)		
	Post-SAH days 0-3	Post-SAH days 5-7	Post-SAH days 10-14
CRP level (mg/L)	106.90 ± 88.9	129.68 ± 96.8	75.09 ± 84.3
WBC count (10 ⁶ /mm ³)	13.81 ± 5.4	12.01 ± 5.0	14.35 ± 6.3
Hgb level (mg/dL)	12.52 ± 1.7	12.10 ± 1.6	10.84 ± 1.2
Fibrinogen (mg/dL)	415.61 ± 163.0	617.63 ± 241.1	600.33 ± 247.7
sRAGE (pg/mL)	3.91 ± 4.0	4.24 ± 3.9	4.05 ± 3.8
Treatment outcome (according to GOS at 3 months)			
Low disability (score of 5)	5 (19%)		
Moderate disability (score of 4)	2 (7%)		
Severe disability (score of 3)	4 (15%)		
Persistent vegetative state (score of 2)	5 (19%)		
Death (score of 1)	11 (41%)		

TABLE 2: Spearman's correlation between treatment outcome and monitored parameters.

	Parameter	Correlation coefficient	<i>p</i> value
Days 0-3 post-SAH	CRP	-0.273	0.257
	WBC	-0.433	0.063
	Hgb	0.118	0.628
	Fibrinogen	-0.286	0.248
	sRAGE	-0.177	0.454
Days 5-7 post-SAH	CRP	-0.339	0.198
	WBC	-0.333	0.207
	Hgb	0.001	0.995
	Fibrinogen	-0.484	0.057
	sRAGE	-0.302	0.254
Days 10-14 post-SAH	CRP	-0.480	0.134
	WBC	-0.086	0.800
	Hgb	0.493	0.122
	Fibrinogen	-0.626	0.070
	sRAGE	0.139	0.682
	WFNS on admission	-0.741	<0.001
	HH on admission	-0.656	<0.001
	GCS on admission	0.688	<0.001

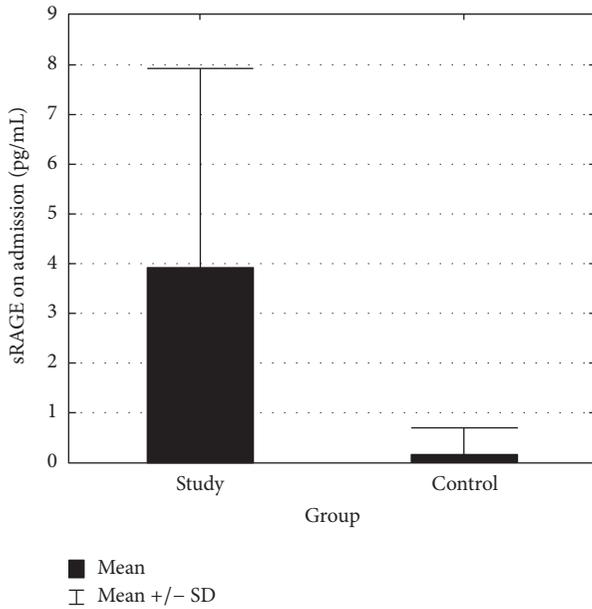


FIGURE 1: sRAGE level in study and control group. Mann-Whitney test revealed significantly higher ($p < 0.001$) levels of sRAGE in CSF of SAH patients.

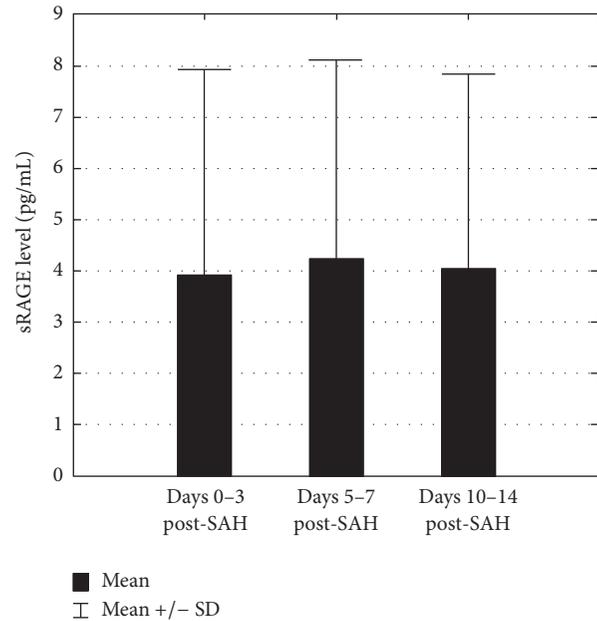


FIGURE 3: sRAGE level changes in time in study group. Skillings-Mack test indicates no significant change of CSF sRAGE level during hospitalisation ($p = 0.868$).

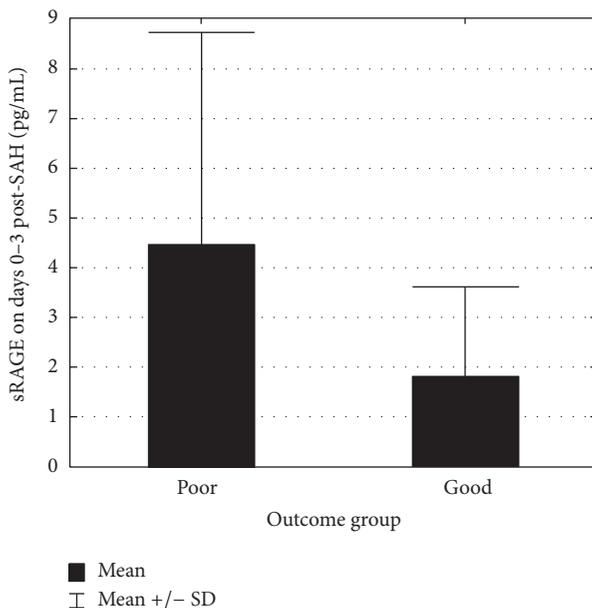


FIGURE 2: sRAGE level in patients with poor and good treatment outcome on days 0-3 post-SAH. Mann-Whitney test revealed no significant difference ($p = 0.234$) in sRAGE level between patients with good and poor treatment outcome.

4. Discussion

Our study demonstrated elevation of CSF sRAGE in patients with poor grade SAH requiring EVD insertion. Clinical studies of sRAGE in patients with neurological disorders have thus far revealed the following: (1) serum sRAGE elevation in ischaemic stroke patients [18], (2) serum sRAGE

correlation with severity of the axonal subtype of Guillain-Barré syndrome [19], and (3) CSF sRAGE decrease in patients with multiple sclerosis and Guillain-Barré syndrome [19, 20]. We are not aware of any previous reports demonstrating elevation of sRAGE in CSF following pathological processes, particularly in patients with SAH.

RAGE is a transmembrane protein that belongs to the immunoglobulin superfamily [18, 23-25]. The human RAGE gene is located on chromosome 6 and its expression leads to production of a 55-kDa type I membrane glycoprotein [24]. Soluble isoforms of RAGE are formed either by (1) removal of the transmembrane region from the pre-RNA during alternative splicing (leading to the production of endogenous sRAGE) or (2) proteolytic cleavage of the full-length membrane form of RAGE protein (mRAGE) by a membrane metalloproteinase called ADAM 10 or an extracellular matrix metalloproteinase 9 (MMP-9) [25-27]. ADAM 10 is a representative of sheddases, membrane-bound enzymes that cleave extracellular portions of transmembrane proteins, releasing the soluble ectodomains from the cell surface. In healthy population, mean blood plasma sRAGE concentration ranges from 800 to 1500 pg/mL [28, 29]. In our study, CSF sRAGE levels in control patients without a history of neurological disorder were undetectable. This finding is in accordance with the recent findings of Zhang et al. [19]. A rat experimental SAH model revealed significant increases in RAGE protein and mRNA levels in neurons and microglia [30]. Furthermore, an increase of MMP-9 levels in both CSF and serum was observed during SAH [31]. Based on these findings, we suspect that three mechanisms are leading to an increase of sRAGE levels in the CSF in our patients. Firstly, SAH-induced expression of RAGE leads

to overexpression of all its isoforms, including endogenous sRAGE. This explanation follows Tang et al. hypothesis that high levels of plasma sRAGE at 48 h after stroke may reflect the rapid activation of mRAGE expression induced by the cerebral ischaemia [18]. A second possible mechanism is an excessive cleavage of membrane-bound RAGE. Increase of RAGE expression on cell membranes and rise of MMP-9 level (both observed during SAH) support this hypothesis [30, 31]. A third expected mechanism is introduction of free plasma sRAGE during aneurysm rupture and blood extravasation to subarachnoid space. Estimated total SAH volume equals 35 mL and as a blood contains 200 to 400 times higher sRAGE levels than those measured in CSF, we would expect more significant elevation of sRAGE levels originating from the SAH patients analysed in our study [32].

As our understanding of the SAH complications has improved, identifying mediators of its critical pathways and designing new targeted therapies becomes of primary importance in neuroproteomics research [33]. The inflammatory reaction, which contributes to SAH-induced brain injury is characterised by complex, multilevel interactions between its separate components. The activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) observed in SAH leads to excessive inflammation and subsequent brain injury [34, 35]. NF- κ B activation is mediated by numerous upstream pathways, including those starting at RAGE and Toll-like receptors 2 and 4 (TLR2/TLR4). Results of our previous studies on soluble TLR2 and 4 suggested that these played only a minor role in this inhibitory mechanism [36]. Tang et al. in their study of sRAGE in human stroke patients found sRAGE to be an independent predictor of functional outcome. In experimental settings, administration of recombinant sRAGE significantly improved the outcome after ischaemic stroke in mice. The suspected protective mechanism depends on high mobility group box 1 binding [18]. Quade-Lyssy et al. have reported that atorvastatin increased the levels of serum sRAGE [37], whilst Cheng et al. [38] and Potey et al. [39] have shown some evidence that atorvastatin ameliorates vasospasm and EBI after SAH. However, the STASH failed to detect any benefit to the long- or short-term outcome using simvastatin in aneurysmal SAH [40]. In the most recent report by Wang et al., administration of recombinant sRAGE significantly reduced the number of positive TUNEL staining cells in SAH rat and improved cell viability in post-SAH CSF-treated cultured neurons [41]. In our study, sRAGE levels failed to differentiate between good and poor outcome patients. These preliminary findings are similar to the results obtained with soluble TLR2/TLR4 and might suggest that sRAGE has limited significance as a prognostic biomarker. Yet, further investigations (addressing limitations of the current study) are essential to assess role of sRAGE in the endogenous anti-inflammatory mechanism. Our study showed no correlation between sRAGE and systemic inflammatory mediators. Although CRP is able to augment mRNA expression of RAGE genes, the levels to which this can be achieved are not known [42].

Conclusions are limited by the small number of patients involved in the study and lack of consecutive sampling in most cases. The enrolled patients do not represent the

full spectrum of SAH as hydrocephalus was an inclusion criterion and could have contributed to brain injury before EVD insertion. Despite careful monitoring, EVD infection remains a potential bias. In addition, only a few elements of the inflammatory pathway were investigated, and further investigation will be required to elucidate the larger picture of post-SAH inflammation.

5. Conclusions

CSF levels of sRAGE increase early in patients with SAH who require acute treatment of hydrocephalus and remain elevated but do not correlate with treatment outcome. The significance of sRAGE as an endogenous anti-inflammatory mechanism requires further investigation.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. Received Funding no. DI2014 005044 did not lead to any conflicts of interest regarding the publication of this manuscript.

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Review Article

Treatment of Cervical Artery Dissection: Antithrombotics, Thrombolysis, and Endovascular Therapy

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Cervical artery dissection (CAD) is an important cause of stroke for young patients, accounting for 5–22% of strokes in patients <45 years of age, which presents not only a great burden to the stroke victims but also a financial burden to the family and society. Because CAD can lead to different clinical lesions, including neuropathy, acute ischemic stroke, and subarachnoid hemorrhage, and is an arterial dissection with a self-healing tendency, the treatment options depend on the clinical manifestations. The main purpose of the treatment is to control CAD-induced neuronal damage and to restore blood flow. The treatment programs include drug treatment and endovascular treatment. However, antithrombotic treatment is crucial. Both antiplatelet drugs and anticoagulant drugs are used to reduce the risk of stroke, but whether one treatment strategy is more effective than the other is unknown. The efficacy and timing of the endovascular treatment of CAD remain controversial.

1. Introduction

During cervical artery dissection (CAD), arterial blood enters the blood vessel wall through the damaged carotid intima, which separates the intimal and media layers. This process causes the formation of an intramural hematoma, resulting in stenosis or occlusion. CAD can cause thrombosis and vascular stenosis and is the major cause of stroke among young people. Patients with CAD as the main cause account for 2.5% of all stroke patients and 5–22% of young stroke patients below 45 years of age [1]. Recently, the prevalence of CAD has attracted increasing clinical attention due to the continuous development of imaging technologies. However, the pathogenesis of CAD is still unclear. Despite the fact that treatment options for stroke remain limited [2], disputes on the efficacies of anticoagulation and antiplatelet therapies are increasing, and the efficacy and timing of the endovascular treatment of CAD remain controversial. The aim of this review is to summarize the recent reported results regarding the treatment of CAD.

2. Diagnosis of CAD

Mostly CAD patients are young; according to studies in Europe and America, the average age of CAD patients is 44.0–45.8 years [3]. A European hospital-based multicenter study showed that males accounted for 53–57% of CAD patients, whereas a North American population-based study revealed that females accounted for 50–52% of CAD patients [4, 5]. The clinical manifestations of CAD are diverse, with typical features termed the CAD triad (ipsilateral pain in the head, neck, and face, Horner syndrome, and cerebral or retinal ischemic symptoms) [6]. However, less than one-third of patients manifest the triad, and the most common symptoms are headache (70–80%) and cerebral ischemic symptoms (67%) [7]. Notably, approximately 5% of CAD patients are asymptomatic [8]. Ischemic stroke is the most common type of secondary brain vascular disease in CAD patients. Due to the continuous development of medical imaging technologies, the CAD diagnosis largely depends on imaging techniques, such as computed tomography angiogram

(CTA), magnetic resonance imaging (MRI), digital subtraction angiography (DSA), and Doppler ultrasound. Of these techniques, DSA has long been considered as the gold standard for CAD diagnosis. In DSA, artery dissection exhibits beaded and thread-like symptoms, irregular fan-shaped stenosis, indirect signs, such as pseudoaneurysm and venous phase contrast agent retention, and direct signs, such as dual chamber symptoms of two-way blood flow [9]. Due to its wide application and noninvasiveness, CTA can provide important information for the diagnosis of CAD. CTA has a false positive rate of 0 for the diagnosis of vascular occlusion and a detection rate of 96% for the diagnosis of vessel wall thickening and irregular changes and is superior to MRI in revealing intimal flaps and pseudoaneurysms [10]. Color Doppler ultrasound can directly show the situation of the arterial wall and detect both direct and indirect signs of CAD. Transcranial Doppler (TCD) is capable of measuring the blood flow velocity and performing arterial emboli monitoring and helps to determine the presence of CAD. In severe carotid artery stenosis or occlusion, the sensitivity of ultrasound can be 100%, whereas, in mild stenosis, the sensitivity drops to 40% [11]. Currently, noninvasive imaging techniques, such as MRI and magnetic resonance angiography (MRA), are playing increasingly important roles in the CAD diagnosis. MRI diffusion-weighted imaging (DWI) can lead to the early detection of CAD-induced cerebral changes. Axial MRI can show the situation on the blood vessel wall or lumen to some extent [12]. MRA experiences less interference from the bone structure and more completely displays vascular structures, especially in the presence of a contrast agent. High-resolution imaging of the vascular wall structure presented by high-resolution MRI (HRMRI) can differentiate the carotid artery and the surrounding tissues, such as the vertebral artery and the surrounding veins, and is more conducive to the identification of a vessel wall hematoma and intravascular thrombus [10].

3. Anticoagulant Therapy and Antiplatelet Therapy of CAD

Drug treatment primarily consists of antithrombotic therapy (i.e., anticoagulant and antiplatelet therapies). Anticoagulant therapy includes intravenous heparin therapy coupled with oral application of warfarin, whereas antiplatelet therapy includes a single oral antiplatelet treatment with one antiplatelet aggregation drug or a dual antiplatelet treatment with a combination of two antiplatelet aggregation drugs as the main treatment programs; antiplatelet treatment includes aspirin, dipyridamole, or clopidogrel alone or in combination.

The main rationale for antithrombotic therapy is that transcranial Doppler studies have demonstrated that the frequency of intracranial microembolic events is rather high in CAD patients [10]. After a stroke, antithrombotic therapy needs to be performed immediately to minimize the thrombosis at the dissection site and to reduce CAD-induced neuronal damage. The duration of antithrombotic therapy is generally 3–6 months, and this therapy rarely lasts more than 6 months. There are no clear guidelines regarding the sign

for the termination of antithrombotic treatment; generally, vascular imaging characteristics, such as dissection healing or vascular occlusion, are used to develop further therapy programs after antithrombotic treatment [13, 14].

In 2011, the US extracranial carotid and vertebral artery disease management guidelines recommended that symptomatic CAD patients be subjected to oral anticoagulant therapy with warfarin (INR2.0~3.0) for 3 to 6 months after intravenous heparin and then switched to long-term use of aspirin or clopidogrel antiplatelet therapy [15].

At present, there has been a lack of large randomized controlled trials to compare the efficacies of antiplatelet and anticoagulant drugs for the prevention of recurrent stroke in CAD patients. Clinicians should choose treatment options based on personal experience and the patient's specific circumstances. Kennedy et al. conducted a meta-analysis in 2012 in which 40 nonrandomized groups consisting of 1636 cases were analyzed. The results showed that, for the treatment of recurrent stroke risk with antiplatelet and anticoagulant therapies, the outcome was as follows: antiplatelet aggregation drugs 2.6% (13/499) versus anticoagulation drugs 1.8% (20/1137), odds ratio (OR) 1.49, whereas, for the risk of death, the outcome was as follows: antiplatelet aggregation drugs 1.00% (5/499) versus anticoagulation drugs 0.80% (9/1137), OR1.27. However, the differences were not significant, and there was no clear evidence that either anticoagulants or antiplatelet aggregation drugs had an obvious advantage [16]. A meta-analysis conducted by Sarikaya et al. in 2013 obtained similar results, but the authors noted that more emphasis should be placed on the use of antiplatelet aggregation drugs because of their convenience and cost and that they should be recommended as the first-line medication [17]. The Cervical Artery Dissection in Stroke Study (CADISS) was a multicenter prospective randomized controlled study in which the efficacy and safety of antiplatelet and anticoagulant therapies in patients with an acute CAD onset within 7 days were investigated. Rigorous randomized controlled experiments on 250 cases found no significant differences in the efficacies of the anticoagulation and antiplatelet therapies; only 2% of patients had a stroke incidence, which was lower than the incidence reported in other observational studies. However, the study had some limitations; for example, the follow-up time was only three months, the long-term efficacy was not followed up, and the sample size was inadequate. Therefore, the study could not determine the difference between the two treatments [18]. Still, during the study, the researchers conducted an analysis using data from the patient population unsuitable for randomization (CADISS.NR) and a meta-analysis on 40 nonrandomized items, including the CADISS.NR research. The results showed that 499 of the 1636 cases received antiplatelet therapy and 1137 received anticoagulation therapy; the recurrent stroke rates were 2.6% and 1.8% for these two groups and the fatality rates were 1% and 0.8%, respectively. No significant differences were detected between the two treatments [19].

In-depth study of the pathogenesis showed that, in addition to secondary hypoperfusion and an arterial originated embolism caused by thrombosis shedding in the dissection, hemodynamic instability played an important role in

the CAD occurrence of intramural hematoma formation [20, 21]. Thus, the use of anticoagulant drugs might cause intramural hematoma expansion and exacerbate abnormal dissection hemodynamics. Moreover, the recurrence rate of CAD-caused ischemic stroke is low, and the persistent risk of bleeding during anticoagulation therapy to some extent offsets the benefits of the anticoagulation therapy. The significance of antiplatelet therapy lies in the prevention of the recurrence of early stroke. Based on clinical experience, the application of antiplatelet therapy has a wider range, including stenosis, occlusion, and pseudoaneurysm. The use of antiplatelet aggregation drugs is also recommended in CAD patients with a poor prognosis or a large number of embolism incidences [13, 22]. Borgess type I and II patients all benefited from dual antiplatelet therapy [23]. Thus, drug treatment programs for patients can be determined by taking into account the following points: (1) anticoagulant therapy should be preferred for CAD patients in the acute stage (within 7 days of the onset) with obvious symptoms (i.e., after intravenous heparin, switch to anticoagulation treatment with oral warfarin (INR2.0~3.0), for 3–6 months); however, antiplatelet therapy needs to be immediately terminated for patients with severe stroke (NIHSS score ≥ 15) complicated with intracranial atherosclerotic disease (ICAD) or local compression symptoms not complicated with stroke/TIA (transient ischemic attack), complicated with diseases with a high risk of bleeding, and complicated with factors such as poor intracranial collateral circulation [13]; (2) based on the extensiveness and the safety of the drug use, antiplatelet therapy should be preferentially adopted for patients with other types of CAD, with dual antiplatelet therapy for 3 months considered appropriate; and (3) quality of life should be improved to control other risk factors.

4. Thrombolysis Therapy of CAD

Intravenous thrombolysis is an effective treatment for ischemic stroke [24]. The treatment of acute cerebral infarction using recombinant tissue-type plasminogen activator (rtPA) has proven to be effective in lowering the mortality and morbidity of acute cerebral infarction in multiple large randomized trials [25, 26]. In CAD-induced ischemic stroke, clinicians are often concerned that rtPA thrombolytic therapy may aggravate vascular injury and increase the risk of bleeding. However, only limited cases have been reported to date, and analyses on the efficacy and safety of thrombolysis therapy in patients with CAD-induced ischemic stroke are lacking from randomized controlled studies. In a recent meta-analysis on patients receiving intravenous thrombolysis and arterial therapies in the Safe Implementation of Thrombolysis in Stroke International Stroke Thrombolysis Register (SITS-ISTR) as of March 2010, 180 cases of CAD patients with acute ischemic stroke (with an average NIHSS score of 16) were investigated, of whom 67% received intravenous thrombolysis therapy and 33% received arterial thrombolysis therapy; the outcome was that the overall incidence of intracranial hemorrhage, the overall mortality rate, and the proportion of patients with a good prognosis were 3.1%, 8.1%, and 41%, respectively. Compared with stroke cases caused by

other etiologies in the SITS-ISTR, the CAD patients receiving thrombolysis therapies showed no significant differences in terms of safety and prognosis [27]. Thus, we believe that the treatment of CAD-induced acute ischemic stroke using intravenous rtPA within 4.5 h of onset is safe. However, we should strive to develop new therapeutic strategies to lower the mortality and disability rates of CAD patients after thrombolytic therapy [25].

5. Endovascular Treatment of CAD

Endovascular treatment has been widely used to treat cardiovascular and cerebrovascular diseases [28]. However, randomized controlled studies on the application of endovascular treatment or surgeries for CAD patients have not been reported to date [29, 30], and the efficacy and safety of endovascular therapy or surgical treatment have not been evaluated in CAD patients. Endovascular treatment has been primarily used in CAD patients with failed antithrombotic treatment with contraindications for anticoagulation and a pseudoaneurysm and when stent implantation is the main vascular interventional procedure. Due to the special pathological physiology of cervical artery dissection, the method of endovascular treatment is cervical artery stenting. Endovascular treatment/surgical treatment for CAD should be limited because CAD patients have a lower risk of recurrent ischemic stroke, there is no significant correlation with CAD-induced vascular stenosis and pseudoaneurysm, and endovascular/surgical treatments are traumatic. With the development of vascular interventional procedures, the application of endovascular treatment in CAD patients may be underestimated; furthermore, it was previously believed that the dissection leads to clinical events mainly through thromboembolism rather than hypoperfusion; thus, antithrombotic therapy has been the preferred treatment for CAD [31]. However, endovascular treatment can also be viewed as the preferred option for the treatment of CAD patients, especially when the patient has both an embolism and obvious hypoperfusion [32]. In this case, endovascular treatment can effectively relieve stenosis, increase blood flow, and improve low perfusion. In a retrospective study, 140 cases of CAD patients received stenting, and angiographic follow-up was conducted for an average of 12.8 months. The results showed that dissection-induced vascular stenosis was significantly improved and that secondary stroke events accounted for only 1.4% of cases. Thus, endovascular therapy could effectively improve CAD-induced vascular stenosis and reduce the incidence of ischemic stroke [33]. Multiple overlapping stents could also effectively reduce the blood flow velocity in pseudoaneurysms and promote thrombosis, thereby shrinking the pseudoaneurysm or causing it to disappear. Previous studies showed that dissection stenosis of CAD patients undergoing stenting therapy could be largely eased, from 71% to complete remission [29]. In terms of the progression of CAD and the structural damage to the vessel wall, patients in the acute stage and Borgess type IB and II patients would significantly benefit from the use of stenting as the preferred treatment [23].

Endovascular treatment also has some specific risks, the most important of which is that the stenting operation

TABLE 1: The summary of treatment of CAD.

Therapies	Indications	Advantages	Disadvantages
Antithrombotics	Conventional therapy in the acute and chronic phase	Oral application and good compliance	Void for part of patients
Thrombolysis	For patients within 4.5 h of onset	Reopen an occluded artery quickly	Maybe leading to intramural bleeding
Endovascular therapy	For patients who have definite recurrent cerebral ischemic events despite medical therapy	Higher rates of revascularization	Potential risks, including peripheral thromboembolism, arterial spasm, and stent thrombosis

Note. CAD: cervical artery dissection.

in winding vessels is prone to some unforeseen outcomes [34]. Complications of endovascular treatment are numerous and range from mild to severe transient neurological damage and even death, including iatrogenic arterial dissection, peripheral thromboembolism, arterial spasm, stent thrombosis, arterial wall perforation by the guide wire, stent migration, stroke, and endometrial hyperplasia [32]. The timing of endovascular treatment for atherosclerosis is now unclear, especially in the case of dissection. In endovascular treatment, an emboli protection device can effectively reduce the risk of embolism during the procedure [35].

Although endovascular treatment has a higher risk and higher requirements on the operator than drug therapy, CAD patients (especially those in the acute stage) would greatly benefit from the strict control of surgical indications. If drug therapy is ineffective for the patient and the patient can generally withstand surgery and is suggested to have acute cerebral infarction by laboratory examination, stenosis, or occlusion caused by hematoma based on the pathophysiological manifestations, or an expanding dissection lesion, the implementation of endovascular surgery would generate more benefits than risks [33].

6. Conclusions and Further Directions

CAD is an important factor that causes stroke in young people. The aim of this review was to summarize the treatment of CAD, and the results are summarized in Table 1. CAD is a disease that has only rarely been diagnosed through autopsy but is now readily diagnosed with the in-depth study of CTA, MRI, and DSA applications. Thus, it is imperative to establish a reasonable and standardized treatment system with few disputes. Because the causes of CAD are not clear, its risk factors need to be discovered. In addition to factors such as high blood pressure and high cholesterol [36], Giossi et al. showed the close correlation between connective tissue abnormalities and the incidence of CAD; interestingly, the association of genetic connective tissue diseases with the occurrence of CAD has not been established [37, 38]. Antiplatelet and anticoagulant therapies currently do not show differences in terms of efficacy, although the cases that have been investigated have been rather limited, and larger scale studies need to be performed. Although the timing of endovascular treatment is still an open question, endovascular treatment will be more widely adopted with

further investigations on CAD's secondary injuries. Moreover, the rational use of antihypertensive drugs to control blood pressure in the normal range and to reduce arterial wall pressure is a necessary intervention [1]. Furthermore, the application of statins for the treatment of CAD needs to be addressed; although this approach lacks relevant case studies, Stein et al. comprehensively analyzed 1560 patients with thoracic aortic aneurysms in 2013 and noted that statins played a positive role in the prognosis of aortic aneurysms [39]. Thus, we propose a bold assumption that statins may also have a positive impact on CAD patients, although this hypothesis requires a theoretical basis through more basic experiments with large sample sizes from multicenter randomized controlled trials. Only in this way can we find conclusive evidence for treatment options and eliminate confusion in the treatment of CAD.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Jing Peng and Zunjing Liu contributed equally to this work.

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Research Article

Management of Spontaneous Subarachnoid Hemorrhage Patients with Negative Initial Digital Subtraction Angiogram Findings: Conservative or Aggressive?

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Background. The ideal management of SAH patients with negative initial DSA findings remains unresolved. **Objective.** (i) To present risk factors, clinical courses, and outcomes in different types of SAH patients with negative DSA findings; (ii) to explore the differences of basal vein between aSAH patients and NASAH patients; and (iii) to evaluate the value of repeated DSA for these patients. **Methods.** All SAH patients with negative initial DSA findings between 2013 and 2015 in our hospital were enrolled and were further categorized as perimesencephalic SAH (PMN-SAH) or nonperimesencephalic SAH (nPMN-SAH). Risk factors, clinical courses, outcomes, and the basal vein drainage patterns were compared. **Results.** A total of 137 patients were enrolled in the present study. The PMN-SAH group had better GOS and mRS values at 1-year follow-up. Moreover, the nPMN-SAH group had a higher rate of complications. The basal vein drainage pattern showed significant difference when comparing each of the NASAH subtypes with aSAH groups. There was a significant higher rate of a responsible aneurysm in nPMN-SAH group upon repeated DSA. **Conclusions.** SAH patients with negative initial DSA findings had benign clinical courses and outcomes. Repeated DSA studies are strongly advised for patients with the nPMN-SAH pattern.

1. Introduction

Spontaneous subarachnoid hemorrhage (SAH), characterized by bleeding into the subarachnoid space in the absence of trauma, is most often caused by the rupture of an intracranial aneurysm [1, 2]. However, according to the previous studies [3, 4], even though there is now widespread use of digital subtraction angiogram (DSA) to aid in the diagnosis of spontaneous SAH, nearly fifteen percent of cases remain idiopathic [4]. This category of SAH has been termed nonaneurysmal SAH (NASAH), which typically follows a benign clinical course and has a generally favorable prognosis when compared with aneurysmal subarachnoid hemorrhage (aSAH) [5, 6].

According to the distribution pattern of the subarachnoid blood, these NASAH patients are usually divided into two subcategories, perimesencephalic (PMN-SAH) and nonperimesencephalic hemorrhage (nPMN-SAH) [7]. Although it is generally recognized that NASAH has a more preferable outcome than aSAH [5], recent studies suggest that the management of the nPMN-SAH subgroup should be more rigorous in light of its more severe clinical courses and outcomes [8, 9]. As nPMN-SAH is a diagnosis of exclusion, some controversial issues remain regarding the management of these patients that have negative initial DSA findings [10, 11]. Despite numerous studies utilizing multiple imaging modalities, the bleeding source of NASAH has not been elucidated. Though most authors support the hypothesis that

the culprit of the bleeding is of venous origin, the precise mechanism of the bleeding source remains unknown [12]. Furthermore, given the low detection rate of a responsible intracranial lesion in NASAH patients, the need for serial DSAs in these cases remains an open question.

Here we retrospectively analyzed the data of all SAH patients with initial negative DSA findings in our center between 2013 and 2015. The risk factors, clinical courses, and outcomes of these patients were evaluated in our study. In addition, the venous drainage patterns in both subgroups were compared. Finally, the necessity of repeated DSA examinations was also evaluated based on our study and the review of related literature.

2. Methods

2.1. Patients and Procedure. The data of patients who presented with SAH between 2013 and 2015 in our center (Second Affiliated Hospital, School of Medicine, Zhejiang University) were retrospectively analyzed. According to standard management of SAH patients [13, 14], all patients were screened by CTA upon admission, followed by emergent DSA examination. There were 164 SAH patients who had an initial CTA that failed to definitively demonstrate a culprit lesion. Of these, 13 were excluded due to a history of traumatic brain injury or another definitive cause of SAH, leaving 151 patients with negative CTA findings. Among them, 14 patients had positive initial DSA results (2 with a perimesencephalic pattern and 12 with a nonperimesencephalic pattern). This left 137 SAH patients with negative initial DSA findings (hereafter referred to as NASAH patients) who were enrolled in the present study. Based on the distribution of the subarachnoid blood, NASAH patients were divided into PMN-SAH ($n = 82$ patients) and nPMN-SAH groups ($n = 55$) [15]. To further exclude intracranial aneurysm as the source of hemorrhage [16], all of them underwent a repeated DSA examination either 10–14 days after admission or one month after discharge. In total, 57 patients agreed to undergo a repeated DSA during hospitalization or follow-up. Four patients were found to have a culprit intracranial aneurysm on the second angiogram, all of whom were patients with the nonperimesencephalic pattern (Figure 1).

The demographic data includes patient sex, age, smoking history, alcohol use, hypertension, diabetes, and history of anticoagulant use. The Glasgow Coma Score (GCS), Hunt-Hess (HH) grade, and the modified Fisher Scale (mFS) were used for evaluation upon admission [5, 33]. The length of hospital stay (LOS) and in-hospital complications, namely, hydrocephalus, cerebral vasospasm, and rebleeding, were compared between the two groups. All patients were followed up by telephone interview or outpatient clinic at three months and one year after discharge. The outcomes were evaluated using the modified Rankin Scale (mRS) and Glasgow Outcome Scale (GOS).

In order to find a potential venous source of bleeding, we compared the basal vein of Rosenthal (BVR) anatomy among the different groups. Excluding 4 patients who were later found to have a definite bleeding source, the venous

configurations of 133 NASAH patients (82 PMN-SAH and 51 nPMN-SAH) were evaluated and compared to a total of 133 consecutive aSAH patients during the same period. The classification of the BVR was performed as described by Ramazan Buyukkaya et al. [12]. Briefly, the drainage pattern of unilateral BVR was divided into three types:

- (1) Type A (normal continuous): a continuous BVR drains mainly into the vein of Galen.
- (2) Type B (normal discontinuous): in a discontinuous BVR, the anterior part drains into the sphenoparietal sinus or cavernous sinus through an uncus vein and posterior part drains to the vein of Galen.
- (3) Type C (primitive variant): this variant drains mainly to the dural sinuses, not via the vein of Galen (i.e., the perimesencephalic veins drain into the superior petrosal sinus or the BVR drains directly into the transverse or straight sinus).

2.2. Statistical Analysis. Categorical variables of different groups, such as the parameter of demographic data, GCS, HH grade, mFS, mRS, and GOS, were compared using the Chi-squared test or Fisher's exact test. Continuous variables, such as the number of patients in each group, were compared using Student's *t*-test. $p < 0.05$ was considered as significant statistical difference.

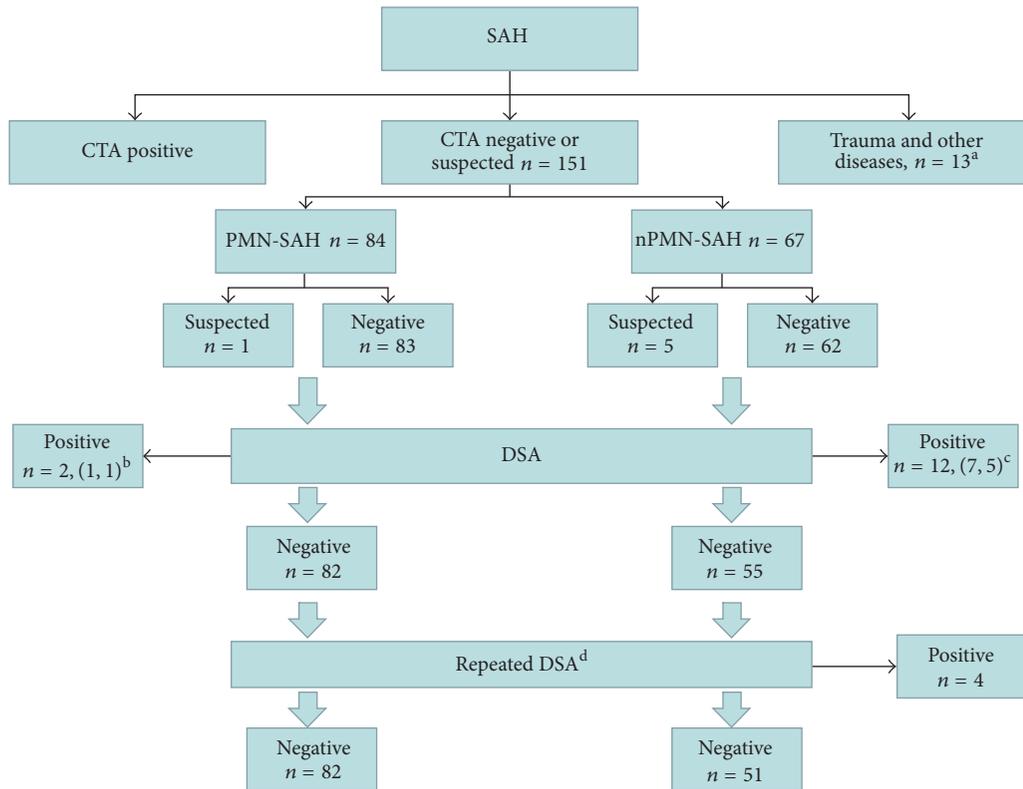
3. Results

3.1. Demographics. The demographics of all NASAH patients with negative initial DSA findings are summarized in Table 1. There were no significant differences in sex, age, smoking history, alcohol abusing history, diabetes, hypertension, or anticoagulation medication history between PMN-SAH and nPMN-SAH groups ($p > 0.05$ for each parameter).

3.2. Clinical Course and Risk Factors for Hydrocephalus. The patients' GCS, HH scale, and mFS were evaluated upon arrival at our emergency room. Despite the interval from ictus to evaluation, the nPMN-SAH group presented with higher GCS and mFS on their initial CT scan ($p < 0.05$). However, there was no significant difference in the HH grade between the two groups ($p = 0.157$) (Table 2).

Complications that developed during hospitalization, hydrocephalus, cerebral vasospasm, and rebleeding were compared. The nPMN-SAH group was associated with higher incidence of hydrocephalus and symptomatic cerebral vasospasm ($p = 0.001$ and 0.017 , resp.). Only one patient experienced rebleeding but no definite source of bleeding was confirmed even though a repeat DSA was conducted. There was no significant difference in the rebleeding rate during hospitalization between the two groups ($p = 0.401$) (Table 2).

There were 14 patients (10.2%) who developed hydrocephalus. The risk factors for hydrocephalus in these patients were compared. There were significant differences in the GCS and mFS between the hydrocephalus group and nonhydrocephalus group ($p < 0.001$). Furthermore, patients who developed hydrocephalus were associated with significantly



a: 13 patients were excluded who were confirmed with head trauma, Moyamoya disease, venous sinus thrombosis, and other diseases.
 b: Initial DSA revealed a responsible aneurysm in 2 PMN-SAH patients, 1 of them was found by the initial CTA.
 c: Initial DSA revealed a responsible aneurysm in 7 nPMN-SAH patients, 5 of them was found by the initial CTA
 d: On repeat evaluation, 4 nPMN-SAH were diagnosed with a responsible intracranial aneurysm.

FIGURE 1

TABLE 1: Demographics of patients with nonaneurysmal subarachnoid hemorrhage.

	NASAH	PMN-SAH	nPMN-SAH	p value
Sum (%)	137 (100%)	82 (59.9%)	55 (40.1%)	NS
Male-female ratio	76/61 (1.2/1)	42/40 (1.05/1)	33/22 (1.5/1)	0.357
Age (range)	56.0 ± 10.4 (30–80)	55.4 ± 9.6 (34–76)	56.7 ± 13.7 (30–80)	0.156
Smoker (%)	18 (13.1%)	11 (13.4%)	7 (12.7%)	0.907
Alcohol abusing (%)	21 (15.3%)	13 (15.9%)	8 (14.5%)	0.835
Diabetes (%)	7 (5.1%)	3 (3.7%)	4 (7.3%)	0.438
Hypertension (%)	30 (21.9%)	15 (18.3%)	15 (27.3%)	0.213
Anticoagulant Using (%)	4 (2.9%)	2 (2.4%)	2 (3.6%)	1

NASAH: nonaneurysmal subarachnoid hemorrhage, PMN-SAH: perimesencephalic nonaneurysmal subarachnoid hemorrhage, nPMN-SAH: nonperimesencephalic nonaneurysmal subarachnoid hemorrhage; NS means can not be analyzed.

higher proportion of a diffuse bleeding pattern on imaging studies (e.g., nPMN-SAH and intraventricular hemorrhage) ($p = 0.002$ and $p < 0.001$, resp., Table 3).

3.3. *Outcome.* A total of 21 patients (12 PMN-SAH and 9 nPMN-SAH) were lost to follow-up after discharge. The mRS and GOS were used to evaluate the outcomes for all patients. We found that patients with PMN-SAH had a better clinical outcome at 3 months after ictus with respect to both mRS

($p < 0.001$) and GOS ($p = 0.003$) (Table 4). Though there was no significant difference of GOS between the two groups at 1-year follow-up ($p = 0.09$), the nPMN-SAH group had a trend towards a higher incidence of mild disability (mRS = 2 or 3, $p = 0.006$, Table 4).

3.4. *BVR Pattern.* The venous phase images on DSA for 3 NASAH patients were unavailable due to technical reasons. The distribution of BVR subtypes of three groups are listed

TABLE 2: The clinical characteristics of nonaneurysmal subarachnoid hemorrhage.

	All NASAH (<i>n</i> = 137)	PMN-SAH (<i>n</i> = 82)	nPMN-SAH (<i>n</i> = 55)	<i>p</i> value
LOS, mean (range)	8.5 ± 8.5 (2–75)	7.7 ± 4.0 (2–16)	12.9 ± 11.9 (2–75)	0.001
GCS				
Mild (13–15)	132 (97.0%)	82 (100%)	50 (90.9%)	0.009
Middle (9–12)	1 (0.7%)	0	1 (1.8%)	
Severe (3–8)	4 (2.3%)	0	4 (7.3%)	
H-H grade				
Good (I-II)	132 (96.4%)	81 (98.8%)	51 (92.7%)	0.157
Poor (III-IV)	5 (3.6%)	1 (1.2%)	4 (7.3%)	
mFS				
0-1	78 (56.9%)	70 (85.4%)	8 (14.5%)	<0.001
2-4	59 (43.1%)	12 (14.6%)	47 (85.5%)	
Complication				
Hydrocephalus	14 (10.2%)	3 (3.7%)	11 (20.0%)	0.001
Cerebral vasospasm	7 (5.1%)	1 (1.2%)	6 (10.9%)	0.017
Rebleeding	1 (0.7%)	0	1 (1.8%)	0.401
Pulmonary infections	3 (2.2%)	1 (1.2%)	2 (3.6%)	0.564

NASA: nonaneurysmal subarachnoid hemorrhage, PMN-SAH: perimesencephalic nonaneurysmal subarachnoid hemorrhage, nPMN-SAH: nonperimesencephalic nonaneurysmal subarachnoid hemorrhage, GCS: Glasgow coma scale, H-H grade: Hunt-Hess grade, mFS: modified Fisher Scale; LOS: length of hospital stay.

TABLE 3: Clinical characteristics of hydrocephalus patients in NASAH group.

	No hydrocephalus (<i>n</i> = 123)	Hydrocephalus (<i>n</i> = 14)	<i>p</i>
Demography			
Age	56.4 ± 10.0	52.9 ± 13.3	0.147
Gender (female)	57 (46.3%)	4 (28.6)	0.205
Smoker	16 (13.0%)	2 (14.3%)	0.893
Alcohol abuse	18 (14.6%)	3 (21.4%)	0.504
Hypertension	26 (21.1%)	4 (28.6%)	0.524
Diabetes	5 (4.1%)	2 (14.3%)	0.100
Clinical grade			
GCS < 13	0 (0%)	4 (28.6%)	<0.001
Modified Fisher Scale > 1	56 (45.5%)	11 (78.6%)	<0.001
Bleed Pattern			
PMN-SAH	79 (63.4%)	3 (21.4%)	0.002
nPMN-SAH	44 (35.8%)	11 (78.6%)	
Anterior circulation ^a	96 (78.0%)	12 (85.7%)	0.506
Posterior circulation ^b	27 (21.9%)	2 (14.3%)	
Intraventricular hemorrhage	8 (6.5%)	10 (71.4%)	<0.001
Cortical hemorrhage	22 (17.9%)	3 (21.4%)	0.745

EVD: external ventricular drain, GCS: Glasgow coma scale.

^aAnterior circulation was characterized by blood mainly locating at anterior of the brain.

^bPosterior circulation was characterized by blood mainly locating at posterior of the brain.

in Table 5. Compared with aSAH patients, there was a significant difference of BVR distribution in both of the PMN-SAH group ($p = 0.003$) and nPMN-SAH group ($p = 0.021$). However, there was no statistically significant difference of unilateral BVR development between PMN-SAH and nPMN-SAH groups ($p = 0.950$). To further analyze the correlation of hemorrhage type and BVR development, the bilateral BVRs were classified as four subgroups, AA,

AB/BB, AC/BC, and CC, respectively. A significant statistical difference was only found between PMN-SAH and aSAH groups ($p = 0.028$, Table 6).

3.5. Repeated DSA Findings. To evaluate the utility of repeated DSA examination in patients with initially negative DSA findings, we compared the detection rate of a responsible intracranial lesion by the repeated DSA in both of

TABLE 4: The prognosis of patient after charged from hospital.

	All NASAH (<i>n</i> = 116)*	PMN-SAH (<i>n</i> = 70)	nPMN-SAH (<i>n</i> = 46)	<i>p</i> value
mRS at 3 months				
0-1	96 (82.8%)	66 (94.3%)	30 (65.2%)	<0.001
2-3	17 (14.7%)	4 (5.7%)	13 (28.2%)	
4-6	3 (2.5%)	0	3 (6.6%)	
GOS at 3 months				
5	110 (94.8%)	70 (100%)	40 (87.0%)	0.003
4	3 (3.1%)	0	3 (6.5%)	
1-3	3 (3.1%)	0	3 (6.5%)	
mRS at 1 year				
0-1	78 (86.7%)	46 (95.8%)	32 (76.2%)	0.006
2-3	10 (11.1%)	2 (4.2%)	8 (19.0%)	
4-6	2 (2.2%)	0	2 (4.8%)	
GOS at 1 year				
5	87 (96.7%)	48 (100%)	39 (92.8%)	0.09
4	1 (1.1%)	0	1 (2.4%)	
1-3	2 (2.2%)	0	2 (4.8%)	

NASA H: nonaneurysmal subarachnoid hemorrhage; PMN-SAH: perimesencephalic nonaneurysmal subarachnoid hemorrhage; nPMN-SAH: nonperimesencephalic nonaneurysmal subarachnoid hemorrhage; GOS: Glasgow outcome scale, mRS: modified Rankin Scale.

*21 patients (12 perimesencephalic and 9 nonperimesencephalic patients) were lost to follow-up after discharge.

TABLE 5: Type of BVR in nonaneurysmal subarachnoid hemorrhage and aneurysmal subarachnoid hemorrhage patients.

BVR type	PMN-SAH	nPMN-SAH	aSAH	<i>p</i> 1	<i>p</i> 2	<i>p</i> 3
Unilateral	<i>n</i> = 160	<i>n</i> = 100	<i>n</i> = 266	0.003	0.021	0.950
A	61 (38.1%)	39 (39.0%)	147 (55.3%)			
B	48 (30.0%)	31 (31.0%)	61 (22.9%)			
C	51 (31.9%)	30 (30.0%)	58 (22.8%)			
Bilateral	<i>n</i> = 80	<i>n</i> = 50	<i>n</i> = 133	0.028	0.066	0.882
AA	15 (18.8%)	9 (18.0%)	50 (37.6%)			
AB, BB	26 (32.5%)	16 (32.0%)	38 (28.6%)			
AC, BC	26 (32.5%)	19 (38.0%)	32 (24.1%)			
CC	13 (16.2%)	6 (12.0%)	13 (9.8%)			

BVR: basal vein of Rosenthal; PMN-SAH: perimesencephalic nonaneurysmal subarachnoid hemorrhage; nPMN-SAH: nonperimesencephalic nonaneurysmal subarachnoid hemorrhage.

*p*1: statistical analysis was proceeded between PMN-SAH and aSAH group.

*p*2: statistical analysis was proceeded nPMN-SAH and aSAH group.

*p*3: statistical analysis was proceeded PMN-SAH and nPMN-SAH group.

the subgroups and also included data from related literature for analysis. A total of 60 patients underwent a repeated DSA examination in our series. On repeat evaluation, 4 of 31 patients with nPMN-SAH were diagnosed with a responsible intracranial aneurysm, resulting in a detection rate of 12.9% on repeat DSA examination; however none of the PMN-SAH patients had positive findings on their repeated DSA (Table 7). According to the data from recent studies, the detection rate of a responsible intracranial aneurysm by repeated DSA was 12.5% in the nPMN-SAH subgroup and 1.2% in the PMN-SAH subgroup (Table 7).

4. Discussion

SAH patients with negative initial DSA findings, usually defined as NASAH, can be a management challenge as

the optimal management scheme for these patients remains controversial. The present study analyzes the data of SAH patients with negative initial DSA findings from our center. We divided those patients into the two subgroups of PMN-SAH and nPMN-SAH. The development of BVR type in each group was compared with an aSAH control group to help elucidate the source of bleeding. The necessity of repeated DSA examinations was evaluated in each group by calculating the positive finding rate from second angiogram.

It is widely accepted that NASAH patients have a more favorable clinical course and a lower incidence of complications when compared to those with aSAH [34]. The subgroup of PMN-SAH was first described by van Gijn et al. [35] as a benign entity, characterized by the distribution of the subarachnoid hemorrhage mainly or only in the perimesencephalic cisterns. However, the nPMN-SAH group has

TABLE 6: Type of BVR in nonaneurysmal subarachnoid hemorrhage and aneurysmal subarachnoid hemorrhage patients.

Author/year	Unilateral BVR type						Bilateral BVR type										
	PMN-SAH (%)			aSAH (%)			PMN-SAH (%)			aSAH (%)							
	A	B	C	A	B	C	AA	AB	BB	AC	BC	AA	AB	BB	AC	BC	CC
Watanabe et al. [17]/2002	3 (25)	2 (17)	7 (58)	79 (41)	70 (37)	42 (22)	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Alén et al. [18]/2008*	36 (24)	66 (44)	47 (32)	116 (59)	51 (26)	31 (15)	12 (13)	41 (45)	38 (42)	54 (48)	34 (30)	24 (21)					
van der Schaaf et al. [19]/2008	21 (19)	48 (43)	21 (37)	49 (58)	26 (31)	9 (11)	4 (7)	NS	NS	29 (53)	NS	NS	NS	NS	NS	NS	8 (19)
Yamakawa et al. [20]/2008	10 (29)	7 (20)	18 (51)	111 (57)	61 (31)	23 (12)	2 (11)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Daenekindt et al. [21]/2008	49 (42)	34 (30)	32 (28)	50 (46)	31 (29)	27 (25)	13 (22)	18 (31)	28 (47)	10 (17)	28 (47)	21 (36)					
Song et al. [22]/2010	NS	NS	NS	NS	NS	NS	10 (31)	8 (25)	8 (25)	6 (19)	34 (60)	12 (21)	9 (16)	2 (4)			
Kawamura et al. [23]/2011	6 (32)	8 (42)	5 (26)	33 (49)	27 (35)	7 (10.4)	0	5 (50)	3 (30)	1 (10)	14 (42)	12 (36)	7 (21)				
Sabatino et al. [24]/2014*	36 (46)	31 (39)	12 (15)	50 (66)	8 (11)	18 (24)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Buyukkaya et al. [12]/2014	NS	NS	NS	NS	NS	NS	14 (35)	3 (9)	12 (34)	6 (17%)	26 (74)	5 (14)	3 (9)	1 (3)			

PMN-SAH: perimesencephalic nonaneurysmal subarachnoid hemorrhage; aSAH: aneurysmal subarachnoid hemorrhage; NS means no data was recorded. *The idiopathic subarachnoid hemorrhage has not been described in detail; no group was divided.

TABLE 7: Diagnostic yield of a repeated DSA investigation.

Author/year	Study type	PMN-SAH	Positive	Misdiagnose rate	nPMN-SAH	Positive	Misdiagnose rate
Topcuoglu et al. [10]/2003	Retrospective	31	0	0	36	3	8.3%
Jung et al. [25]/2006	Retrospective	65	1	1.5%	37	17	45.9%
Huttner et al. [11]/2006	Prospective	38	0	0	NS	NS	NS
Little et al. [26]/2007	Retrospective	23	1	4.3%	59	5	8.5%
Gupta et al. [3]/2009	Retrospective	18	0	0	43	2	4.7%
Carvi y Nievas and Archavlis [27]/2009	Retrospective	8	0	0	3	1	33.3%
Agid et al. [9]/2010	Retrospective	28	0	0	28	4	14.3%
Fontanella et al. [28]/2011	Retrospective	23	0	0	72	9	12.5%
Maslehaty et al. [29]/2011	Retrospective	34	1	2.9%	120	13	10.8%
Kelliny et al. [30]/2011	Retrospective	35	0	0	37	6	16.2%
Delgado Almandoz et al. [31]/2012	Prospective	29	1	3.4%	39	2	5.1%
Lin et al. [32]/2012	Retrospective	27	0	0	41	2	4.9%
DW et al. [8]/2012	Retrospective	6	0	0	12	2	16.7%
Present study	Retrospective	31	0	0	29	4	13.8%
Total		365	4	1.1%	556	70	12.6%

PMN-SAH: perimesencephalic nonaneurysmal subarachnoid hemorrhage; nPMN-SAH: nonperimesencephalic nonaneurysmal subarachnoid hemorrhage; NS means no data was recorded.

a more diffuse distribution of the subarachnoid hemorrhage that is more similar to aSAH [15]. A diagnosis of nPMN-SAH is associated with higher rate of complications, such as hydrocephalus, cerebral vasospasm, and cerebral infarction [5]. Hydrocephalus is one of the most common complications of NASAH, normally identified by symptoms of elevated intracranial pressure (progressive headache, altered sensorium) or findings on head CT [36]. The incidence of hydrocephalus from the present cohort was 14.3%, which is in agreement with previous reports [34]. We also found that, in the nonperimesencephalic pattern, the presence of intraventricular hemorrhage or poorer clinical grade on presentation (GCS < 13 and modified Fisher Scale > 2) were associated with the development of hydrocephalus.

Interestingly, despite the repeated imaging studies that have been performed on NASAH patients, the etiology of bleeding remains obscure. The potential pathogenesis may include a venous system variant [20], capillary abnormality, intracranial basilar dissection, ruptured perforating artery [37], cavernous malformation [38], or capillary telangiectasia [15]. Although both arterial and venous origins for the SAH have been proposed, most of the studies favor a venous source of PMN-SAH [39]. It was first hypothesized by Watanabe et al. [17] that a large portion of the BVRs in PMN-SAH patients vary from a normal configuration with

drainage into dural sinuses instead of the vein of Galen. Similarly, van der Schaaf et al. [19] reported that primitive venous drainage was more common in patients with PMN-SAH and that the venous drainage variation was ipsilateral to the side of bleeding. Most of the subsequent studies supported the theory that the drainage into the deep venous drain system (the vein of Rosenthal variant) was related to the PMN-SAH [12, 18–20, 22–24]. However, a study from Daenekindt et al. [21] suggested otherwise (Table 6), but this may be explained by its relatively small patient series, short follow-up period, or differences in diagnostic criteria. Similar to prior publications, our present study demonstrated a significant difference in BVR anatomy between the PMN-SAH and aSAH groups, regardless of unilateral or bilateral BVR type ($p = 0.003, 0.028$) and that the primitive venous configuration was ipsilateral to the source of the bleeding. However, it remains unclear exactly how the venous drainage variation contributes to PMN-SAH.

However, unlike with the PMN-SAH group, the relationship between venous drainage and nPMN-SAH was rarely described previously. It is generally hypothesized that the nPMN pattern may have resulted from an arterial source to account for its relatively “malignant” clinical presentation and extensive blood distribution into the cisterns and parenchyma. Consequently, this pattern of SAH is also

referred to as aneurysm-like SAH. Interestingly, our study revealed that there was also a significant difference in the unilateral BVR type between the nPMN-SAH and aSAH groups ($p = 0.021$). However, for bilateral venous drainage, there was no significant difference between the two groups ($p = 0.066$). The inconsistent findings between the unilateral and bilateral BVR types could be explained by a contribution of true aSAH that were misclassified as nPMN-SAH because of an initial negative DSA. Although a higher portion of nPMN-SAH with negative initial DSA findings were eventually confirmed to have resulted from ruptured intracranial aneurysms, compared to PMN-SAH, most sources of nPMN-SAH remain idiopathic. Therefore, we conclude that if a ruptured intracranial aneurysm can be absolutely excluded, the bleeding source of a true nPMN-SAH may be similar to that found in PMN-SAH.

Another main concern in the management of NASAH is to evaluate the necessity of repeated DSA examinations for each individual. While DSA is currently the standard method to diagnose an intracranial aneurysm for patients suffering from SAH, there are risks to the procedure. Some studies have shown that catheter angiography has up to a 2.6% risk of permanent neurologic complications in NASAH [40]. Recently, numerous reports have proposed the use of noninvasive techniques to diagnose an intracranial aneurysm, such as CTA and magnetic resonance angiography (MRA) [41, 42]. The accuracy of the DSA result can be affected by numerous factors: the resolution of the DSA device, the quality of the acquired scan, the 3D reconstruction capabilities, the interval between the onset of the symptoms and the examination, and the experience of the technician. Furthermore, a small or dissecting aneurysm, hemorrhage or vasospasm concealing the aneurysm, or technical deficiencies can lead to a false-negative result upon the initial examination [43]. Even though improvements in imaging technology have decreased the incidence of misdiagnosis, there are still nearly 15% of SAH patients who have negative findings upon their initial DSA examination [43, 44]. Since missing an aneurysmal source on DSA would expose patients to the extensive morbidity and mortality of rebleed, most practitioners carry out repeat DSA exams on SAH patients with a negative initial DSA. Consequently, it would be of value to select the specific individuals who would benefit from repeated DSA exams.

According to our present study, a total of 60 patients underwent a repeat DSA examination. Four of 31 patients with nPMN-SAH were ultimately diagnosed with a ruptured intracranial aneurysm, resulting in a detection rate of 12.9% for a repeat DSA. However none of the PMN-SAH patients in our study had positive findings on their repeat DSA. To further address this finding, a thorough review of data from past studies was evaluated. There are 13 studies in the past 15 years that discuss a positive finding on repeat DSA exams in SAH patients with negative initial DSA findings. For the pooled data of PMN-SAH patients, a repeat DSA detected a culprit aneurysm in only 4 of 151 patients [2, 3, 8, 27, 28, 30, 32] (Table 7) [25, 26, 29, 31]. Combined with our data, the overall misdiagnosis rate is only 1.1%, which is likely lower than the risk of the DSA procedure itself. We therefore propose that it may be acceptable to follow up PMN-SAH patients by

noninvasive image studies rather than DSA. Some authors go further, even raising the possibility that it is reasonable to manage PMN-SAH patients completely with noninvasive cerebral vascular imaging. The preference at our institution is that unless the initial CT scan with high quality vascular imaging is completed within several hours after the onset of symptoms, all PMN-SAH patients need at least one DSA to exclude a ruptured intracranial aneurysm. However, for centers without available neurointerventionalists, the DSA can be delayed because PMN-SAH is usually associated with a benign clinical course and good prognosis. Unlike the extremely low rate of aneurysm detection in PMN-SAH patients, our data revealed that 12.6% of nPMN-SAH patients were ultimately found to have a responsible aneurysm on the second DSA. In previous studies, the misdiagnosis rate of the initial DSA in nPMN-SAH patients varied greatly (misdiagnose rate from 4.7% to 45.9%), which is likely due to several factors, as previously mentioned. After pooling all the published studies, the overall misdiagnose rate was 12.5% (66 out of 527, Table 7), which is similar to the findings at our institution. Therefore, we strongly recommend a repeat DSA examination for patients with nPMN-SAH who had negative initial findings. Additionally, it is advisable to use 3D image acquisition for both internal carotid arteries and the vertebral arteries in the initial and repeat DSA to optimize the chances of finding small lesions. Since the aneurysms discovered on repeat imaging tend to be miniature in size and are found at the bifurcation of small perforating arteries, we also suggest consultation with an experienced neuroradiological or/and neurointervention physician to establish the diagnosis.

5. Conclusion

Managing SAH patients with negative initial DSA findings can be challenging. Based on the results of our present study and a review of the pertinent literature, the PMN-SAH subgroup usually has a benign clinical course and a repeat DSA very seldom reveals a ruptured intracranial aneurysm. More importantly, nPMN-SAH patients are associated with higher complication rate and incidence of a ruptured aneurysm. Therefore, we strongly recommend a repeat DSA in patients with nPMN-SAH pattern on initial imaging.

Conflicts of Interest

The authors report no conflicts of interest concerning the materials or methods used in this study or findings specified in this paper.

Authors' Contributions

Drs Liang Xu and Yuanjian Fang contributed equally to this work.

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Research Article

Optimization of Catheter Based rtPA Thrombolysis in a Novel In Vitro Clot Model for Intracerebral Hemorrhage

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Hematoma lysis with recombinant tissue plasminogen activator (rtPA) has emerged as an alternative therapy for spontaneous intracerebral hemorrhage (ICH). Optimal dose and schedule are still unclear. The aim of this study was to create a reliable in vitro blood clot model for investigation of optimal drug dose and timing. An in vitro clot model was established, using 25 mL and 50 mL of human blood. Catheters were placed into the clots and three groups, using intraclot application of rtPA, placebo, and catheter alone, were analyzed. Dose-response relationship, repetition, and duration of rtPA treatment and its effectiveness in aged clots were investigated. A significant relative end weight difference was found in rtPA treated clots compared to catheter alone ($p = 0.002$) and placebo treated clots ($p < 0.001$). Dose-response analysis revealed 95% effective dose around 1 mg rtPA in 25 and 50 mL clots. Approximately 80% of relative clot lysis could be achieved after 15 min incubation. Lysis of aged clots was less effective. A new clot model for in vitro investigation was established. Our data suggest that current protocols for rtPA based ICH therapy may be optimized by using less rtPA at shorter incubation times.

1. Introduction

Spontaneous intracerebral hemorrhage occurs in 10–15% of all stroke patients and is still a major cause of stroke-related death and disability [1–3]. Optimal therapy is still controversial. Prospective studies and randomized trials have shown equally poor outcome for best medical treatment or open surgery for evacuation of the hematoma, whereas one study suggests that the latter might be beneficial for selected patients with lobar hematomas not deeper than 1 cm from brain surface and a GCS between 9 and 12 [4–8].

In consideration of these results the interest in minimal invasive procedures has grown. Stereotactic frame-based or image-guided frame-less catheter placement and rtPA lysis of ICH have shown to be safe and effective in volume reduction [9–12]. In the past 2 decades this therapy has emerged to

a well-established fast and easy procedure in many neurosurgical units [13]. However rtPA dosing mainly has been based on clinical experience. No published data are available from (i) in vitro or in vivo studies systematically analyzing optimal timing of rtPA administration, (ii) optimal dosing of the drug, and (iii) duration of efficient treatment. Not surprisingly some authors reported adverse events, which might be dose dependent, such as occurrence of an rtPA induced delayed cytotoxic perifocal edema. This was observed in animal models and clinical settings [14–17], while some other studies did not confirm these findings [18, 19]. A trial on minimal invasive ICH lysis using an intralesional catheter is presently conducted [20].

In order to establish optimal dose and timing for rtPA therapy, however high numbers of repetitive investigations are necessary to achieve valid and significant results. Studies

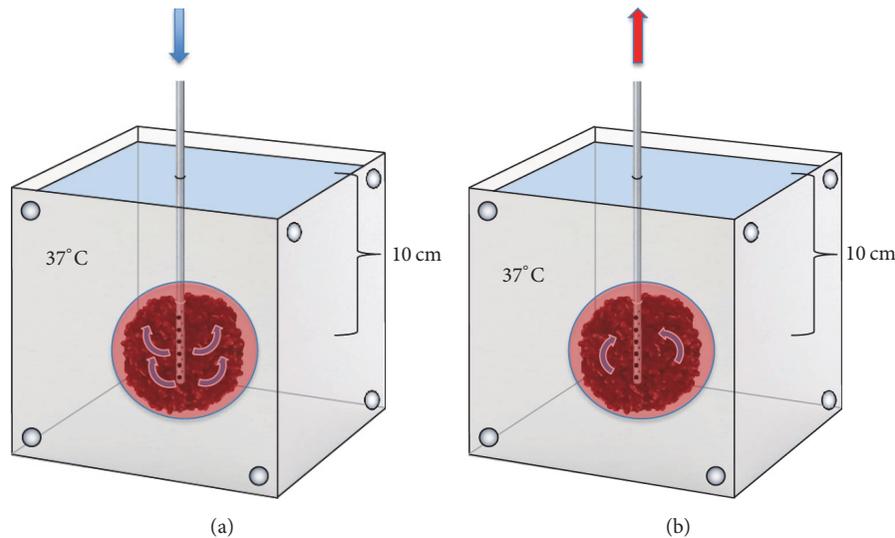


FIGURE 1: Blood clots submerged 10 cm in a 37°C water bath. An EVD is placed in all clots for drug administration (a). After treatment the liquefied fraction is drained by a gravity based drainage system (b).

in large ICH animal models are very expensive. An obvious alternative is an in vitro ICH model, which allows a high number of repetitions under controlled conditions. We here present an easy and robust in vitro ICH model in which we investigated optimal timing and dosing of rtPA lysis [21, 22].

2. Material and Methods

2.1. Blood Clot Preparation. We collected blood from the cubital vein of healthy volunteers into 20 mL syringes (BD Discardit, Germany). In vitro blood clots were produced from 25 mL or 50 mL of human blood supplemented with 10 IE of thrombin (bovine plasma thrombin, Sigma, Germany, final concentration 10 IE/500 μ L) in a balloon tightly closed and incubated 1.5 h in an incubator at 37°C (Heraeus Instruments, Germany). The application of thrombin has been adapted from an intravascular clot model [23, 24]. Before treatment clots were weighed, the clot and serum fraction were separated carefully by a fine mesh and weighed individually and afterwards placed back into the balloon for treatment. After clot production the clots were randomized to the different treatment groups.

2.2. In Vitro ICH Model. An external ventricular drain (EVD) (Neuromedex® GmbH, Switzerland, 9 F, 30 cm length, 20 holes with 1 mm diameter) catheter was placed into all clots, mimicking the intracranial situation of a lysis catheter, and connected to a gravity based EVD drainage system (Neuromedex® GmbH, Switzerland) and placed 10 cm below the blood clot level. The clots were placed 10 cm below surface in a water bath at 37°C. Temperature was constantly monitored by a thermometer (PH Meter, WTW GmbH, Germany) (Figure 1).

After randomization and corresponding to the different experimental protocols the EVD system was opened and the liquefied fraction of the hematoma was drained by gravity.

After treatment the remainder of each clot was weighed to assess the relative weight reduction of the clot.

2.3. Spontaneous Thrombolysis, Carrier Effect, and rtPA Lysis. In the first setting we investigated the amount of spontaneous lysis, a potential carrier effect, and the rtPA lysis effect. Group 1 ($n = 6$, 25 mL clots) was treated with an EVD to drain the liquid fraction after 1 h incubation in a 37°C water bath to quantify the spontaneous lysis process. In group 2 ($n = 6$, 25 mL clots) 5 mL of 0.9% NaCl was administered to the clots. Corresponding to group 1 the drain was opened after 1 h. Three clots (50 mL) were treated with a dose of 3 mg rtPA diluted in a volume of 5 mL in group 3. Drains were opened after 1 h incubation. Total volume of carrier (NaCl) or rtPA was 5 mL to exclude possible effects of different carrier volumes. Following this, relative clot weight reduction was compared for all groups.

2.4. Dose-Response Relationship. A dose-response relationship was evaluated in five groups each consisting of three 25 mL blood clots with five different doses of rtPA (0.5; 0.9; 1.2; 2; 3 mg, treatment time 60 min) using the clot model. Clots were weighed before and after treatment. Similar to this, 5 different doses of rtPA were applied in 50 mL clots (0.5; 0.9; 1.2; 2; 3 mg, treatment time 60 min). Furthermore each rtPA treated clot was compared to a placebo (5 mL 0.9% NaCl) treated clot of the same blood donor. The differences of weight of the treated and the control blood clots were statistically analyzed to assess the lysis effect of each rtPA dose.

2.5. Optimal Treatment Time. In order to investigate the optimal treatment time for rtPA, 25 mL clots were treated with an optimized rtPA dose of 1 mg by different periods of time. After rtPA administration, the EVD system was opened after 5, 15, 30, and 60 min (each time point in replicates

of 3 clots). Relative weight reduction after treatment was compared.

2.6. Effectiveness of rtPA in Different Old Clots. Clots of different ages were produced as described above (1.5 h, 24 h, and 48 h; each group consisting of $n = 3$). One mg rtPA was applied repetitively four times. During each treatment rtPA was applied and remained in the clot for 15 min; then liquid fraction was drained for 10 min. Clots were weighed before and after treatment.

2.7. Statistical Analysis. We summarized results by reporting mean \pm standard deviation. For comparison of spontaneous thrombolysis, carrier effect and rtPA lysis, and effectiveness of 1 mg rtPA in different aged clots statistical analysis was performed by one-way analysis of variance. 95% confidence intervals for all parameters were reported. Two-sided p values below 0.05 were considered as statistically significant. Analysis was performed with SigmaPlot 12.0. (Systat Software, Inc., USA) and GraphPad Prism (version 6.0).

For statistical analysis of the dose-response relationship of rtPA, we fitted a three-parameter logistic model and estimated the 50% and 95% effective dose with 95% confidence intervals based on the fitted model [25].

$$f(x, (b, d, e)) = \frac{d}{1 + \exp\{b(\log(x) - \log(e))\}} \quad (1)$$

Analysis was performed with R software, version 3.0.1: R Core Team (2013), R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, URL <http://www.R-project.org/>). For model fitting the R package DRC was used [25].

3. Results

3.1. Reliability of the Clot Model. A total number of 44 clots of human blood were created. The solid clot and liquid serum parts were separated and weighed. The solid part had an average weight of 21.76 ± 1.03 g and the average liquid serum fraction was 3.81 ± 0.88 g. The low variance in weight shows the consistency of clot formation in this in vitro ICH model (Figures 2(a) and 2(b)) [21, 22].

3.2. Spontaneous Thrombolysis, Carrier Effect, and RtPA Lysis Effect. The control group (drain only) showed a mean relative end weight of $64.96 \pm 5.26\%$ of initial weight. The drain plus carrier treated clots with a mean relative end weight of $69.44 \pm 6.67\%$ showed no significant difference compared to the control group ($p = 0.222$). The rtPA (3 mg) treated clots with a relative end weight of $46.01 \pm 6.1\%$ showed a significant difference compared to control ($p = 0.002$) and to drain plus carrier alone treated clots ($p < 0.001$) (Figure 3) [21, 22].

3.3. Dose-Response Relationship of rtPA in 25 mL Clots. A dose-response relationship was evaluated in five groups each consisting of three 25 mL blood clots using five different doses of rtPA (0.5; 0.9; 1.2; 2; 3 mg) and compared to a placebo (5 mL 0.9% NaCl) treated clot from the same donor. Clots

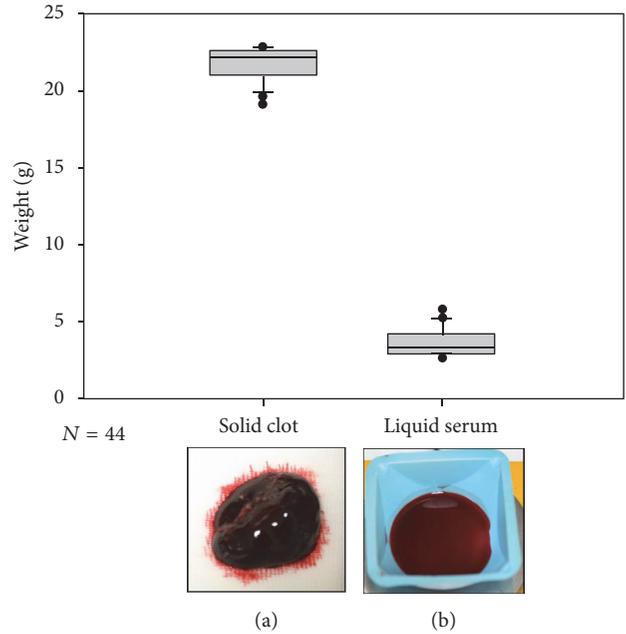


FIGURE 2: Clot formation in vitro; $n = 44$. Weight of clot (21.76 ± 1.03 g) and serum (3.81 ± 0.88 g) part is illustrated in box plots showing the low mean variance. (a) Showing the solid part of the blood clot and (b) the liquid serum part of the clot.

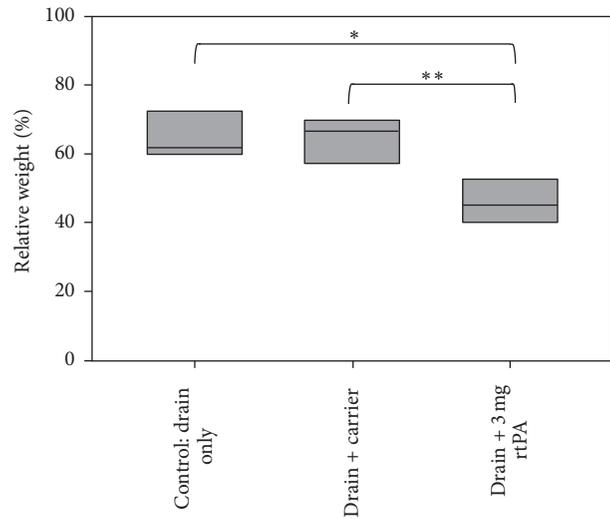


FIGURE 3: Comparison of spontaneous thrombolysis, carrier effect, and rtPA lysis as box plots ($n = 6$). The control group: blood clots treated with drain only had a relative weight of $64.96 \pm 5.26\%$. Blood clots treated with 5 mL of carrier solution: 0.9% NaCl had a relative weight after treatment of $69.44 \pm 6.67\%$. Clots treated with 3 mg of rtPA showed a relative weight of $46.01 \pm 6.1\%$. $***p < 0.001$; $*p = 0.002$.

were weighed before and after treatment. The rtPA treated group showed a relative posttreatment weight of $60.03 \pm 1.76\%$ when treated with 0.5 mg rtPA, $49.06 \pm 0.98\%$ when treated with 0.9 mg rtPA, $48.78 \pm 2.14\%$ when treated with 1.2 mg rtPA, $46.34 \pm 4.68\%$ when treated with 2 mg rtPA, and $46.01 \pm 6.1\%$ when treated with 3 mg rtPA. The control group had a

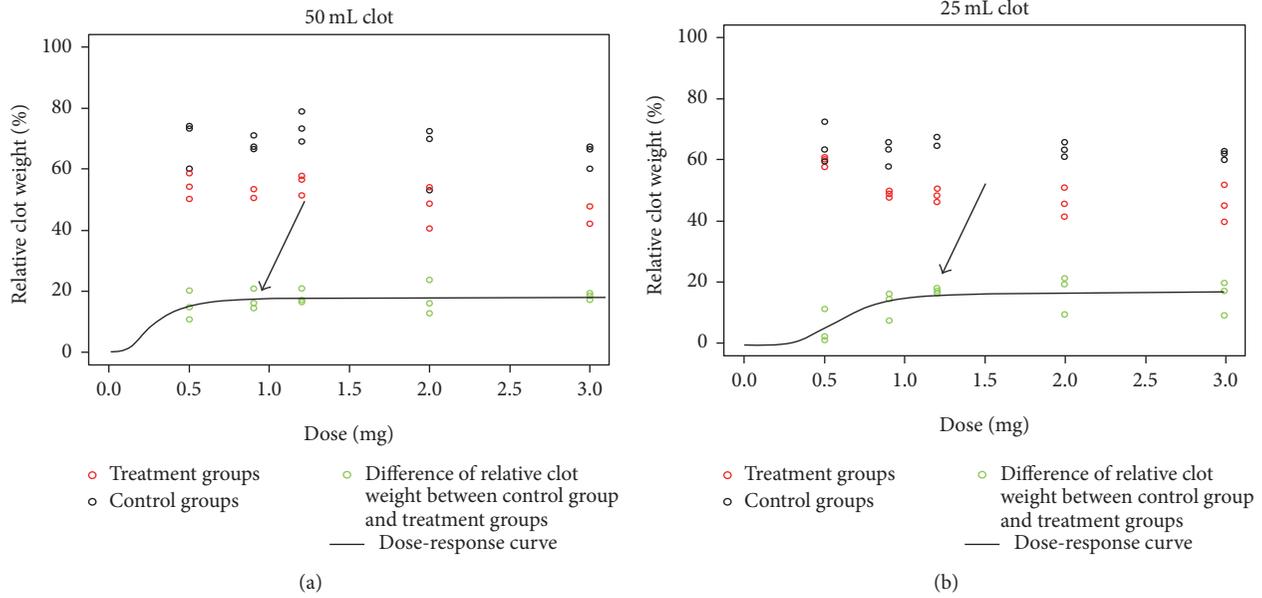


FIGURE 4: (a) A dose-response relationship was evaluated in five groups each consisting of three 25 mL blood clots with five different doses of rtPA (0.5; 0.9; 1.2; 2; 3 mg): treatment group (red) and control group (black). The relative posttreatment clot weight is shown on the y-axis. The differences of weight of the treated and the control blood clots showed the effect of lysis of each rtPA dose (green). The black line shows the dose-response relationship of rtPA. The arrow indicates the 95% effective dose of 1.2 ± 0.52 mg rtPA. (b) Similar to (a) a dose-response relationship was evaluated for 50 mL blood clots in five groups each consisting of three 50 mL blood clots with five different doses of rtPA (0.5; 0.9; 1.2; 2; 3 mg): treatment group (red) and control group (black). The relative posttreatment clot weight is shown on the y-axis. The differences of weight of the treated and the control blood clots showed the effect of lysis of each rtPA dose (green). The black line shows the dose-response relationship of rtPA. The arrow indicates the 95% effective rtPA dose of 0.84 ± 1.07 mg rtPA.

mean relative posttreatment weight of $64.04 \pm 3.67\%$. The 95% effective dose (ED95) was 1.2 ± 0.52 mg rtPA; the 50% effective dose (ED50) was 0.6 ± 0.1 mg (Figure 4(a)) [21, 22].

3.4. Dose-Response Relationship of rtPA in 50 mL Clots. The same experiment was performed in 50 mL clots. The treatment group showed a relative posttreatment weight of $54.23 \pm 4.41\%$ treated with 0.5 mg rtPA, $51.41 \pm 1.79\%$ treated with 0.9 mg rtPA, $55.23 \pm 3.36\%$ treated with 1.2 mg rtPA, $47.91 \pm 6.96\%$ treated with 2 mg rtPA, and $45.98 \pm 3.06\%$ treated with 3 mg rtPA. The control group had a mean relative posttreatment weight of $68.94 \pm 6.52\%$. The 95% effective dose (ED95) was 0.84 ± 1.07 mg rtPA; the 50% effective dose (ED50) was 0.28 ± 0.35 mg (Figure 4(b)) [21, 22].

3.5. Optimal Treatment Time and Lysis Rate. Assuming a maximum lysis rate of 100% after 1 h, lysis rates after different exposure times to rtPA were analyzed in 25 mL clots. The normalized rate of lysis after 5 min exposure to rtPA was $53.22 \pm 3.9\%$; after 15 min it was $79.41 \pm 1.7\%$ and after 30 min $85.38 \pm 1.5\%$ (Figure 5) [21, 22].

3.6. Effectiveness of rtPA in Clots of Different Age. Fibrinolytic treatment with rtPA is less effective in aged clots. There was a significant weight difference of 90 min old clots compared to 24 h ($p < 0.0001$) and 48 h old clots ($p = 0.0002$) during the first treatment (Table 1). During the second treatment

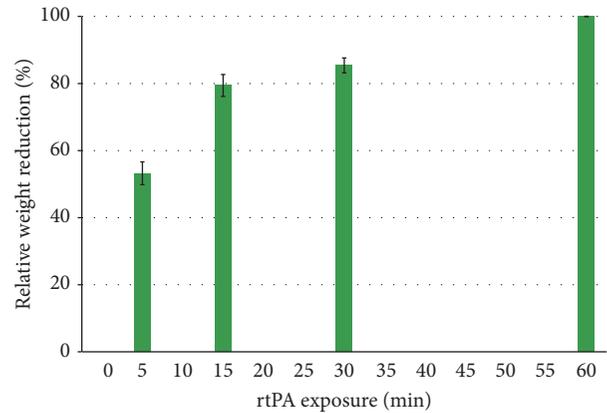


FIGURE 5: The graph shows normalized relative end weights after different time exposures of the blood clots to rtPA for 5, 15, 30, and 60 min determining that 100% lysis takes place within 60 min. Error bars represent standard error per condition; values are rescaled for 100% per 60 min; app. 80% lysis happens during 15 min. Each bar represents $n = 3$ clots.

there was still a significant difference between the 90 min aged clots compared to 24 h aged clots ($p = 0.0059$) (Table 1). Repetitive rtPA treatment showed decreasing effectiveness in weight reduction. The bulk weight reduction was achieved by the first two treatments (Figure 6) [21, 22].

TABLE 1: Relative weight after repetitive treatment with 1 mg rtPA in different aged clots: 90 min, 24 h, and 48 h.

Treatment cycles	90 min	24 h	48 h	Statistics
1	55.23 ± 7.13%	77.78 ± 8.2%	75.71 ± 2.35%	90 min versus 24 h: $p < 0.0001$ 90 min versus 48 h: $p = 0.0002$
2	44.23 ± 5.04%	59.02 ± 4.78%	54.83 ± 7.83%	90 min versus 24 h: $p = 0.0059$
3	41.62 ± 3.11%	49.89 ± 5.65%	48.07 ± 5.17%	
4	34.28 ± 2.33%	44.69 ± 9.77%	44.54 ± 5.86%	

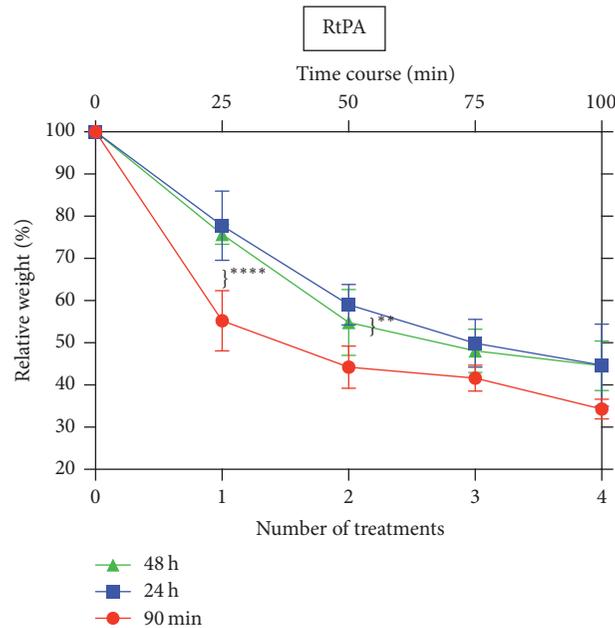


FIGURE 6: Effectiveness of 1 mg rtPA in different old clots: 90 min (red), 24 h (blue), and 48 h (green): the graph shows the relative weight in percent (y -axis) after 1 till 4 treatment cycles (x -axis) ($n = 3$). On top axis the time course shows cycles of 25 min, consisting of 15 min of rtPA-exposure and 10 min drainage period. **** indicates the significant weight difference of 90 min old clots compared to 24 h ($p < 0.0001$) and 48 h old clots ($p = 0.0002$) during the first treatment. ** indicates the significant weight difference of the 90 min old clots compared to 24 h old clots ($p = 0.0059$) during the second treatment.

4. Discussion

In the present study to the best of our knowledge we investigated for the first time a systematic analysis of fibrinolytic therapy of rtPA (supplemental flowchart illustrates the experimental work; see Supplementary Material available online at <https://doi.org/10.1155/2017/5472936>). We established a novel in vitro ICH clot model [21, 22]. 44 blood clots were produced from 25 or 50 mL of human blood from healthy volunteers. The supplementary use of thrombin according to published methods of in vitro micro intravascular clots stabilized the clot independent from donor [23, 24]. The model proved to be highly reproducible in terms of clot formation of solid hematomas and serum fraction (Figure 2) [21, 22]. This setting allowed an easy workflow and a high number of repetitions under controlled conditions and avoided the need of a large ICH animal model [26].

In the first experimental series we evaluated the effect of rtPA lysis in comparison to control groups. Our findings

indicate that normal saline irrigation of clots has no significant fibrinolytic effect [21, 22]. The rtPA treated clots in contrast had a significant loss of their relative end weight, confirming that fibrinolysis takes place in this experimental in vitro setting (Figure 3). These results demonstrating an app. 55% volume reduction after a single dose of 3 mg rtPA are in line with several experimental and clinical studies investigating the fibrinolytic potential of rtPA in ICH [9–19, 26–28]. In one of the first clinical series reported already 2 decades ago Lippitz et al. reported on fibrinolytic therapy after initial stereotactic aspiration of the hematoma. In 10 patients the authors yielded about 60% volume reduction by aspiration and a total of 84% hematoma removal after additional repetitive rtPA administration of 3 mg daily over 1–3 days [9]. The latest published study, an analysis of a Phase II trial patient collective for minimal invasive surgery with rtPA, showed that this therapy is well tolerated and effective. The authors even found a decrease of perihematoma edema

in the rtPA and aspiration treated group compared to the aspiration only treated group. They assume no neurotoxic rtPA effects on perifocal brain tissue when it is administered into the clot. Moreover the larger hematoma volume and reduction of toxic metabolites in the aspiration and rtPA group may lead to a decreased perifocal edema [19]. Until now no clear correlation of rtPA doses and the occurrence or size of a perifocal edema has been published. Interestingly the animal and clinical studies reporting of rtPA related perifocal edema applied relative high cumulative doses of rtPA [14, 16, 17, 26]. The ongoing phase III MISTIE trial will reveal possible dose-related side effects.

To further characterize the optimal dose of rtPA in different clot sizes, we performed a dose-response analysis in 25 mL and 50 mL blood clots. The 95% effective dose of rtPA in 25 mL clots was 1.2 ± 0.52 mg and 0.84 ± 1.07 mg in 50 mL clots [21, 22]. We interpreted the lower dose in larger clot as not significant. In the Phase II MISTIE trial the rtPA dose was evaluated in 2 arms, while arm 1 received 0.3 mg every 8 hours, up to 9 times; arm 2 received 1 mg every 8 hours and up to 9 times in 72 hours. The investigators chose these dose regimens with reference to the recommendation of the American Heart Association in the application of intravenous rtPA and by the experiences of different clinical centers [9–14]. Some authors applied 1 mg rtPA per 10 mL hematoma, whereas hematoma volume was assessed by the formula $A \times B \times C/2$. The total rtPA doses ranged in these series depending on hematoma volume from 5 to 16 mg [10, 14, 15]. In our in vitro study surprisingly a larger clot did not require a higher rtPA dose. This might result from the relative hematoma surface, which can be reached by rtPA molecules per administration via the EVD. Possibly the catheter perforations and design play a role in this phenomenon. However the relative activity of rtPA in 1 mg rtPA seems to be sufficient or excessive even for larger hematoma volumes like 50 mL [21, 22]. The effect of repetition and timing remain unclear.

After assessment of an optimal rtPA dose we investigated the optimal exposure time of the clot to rtPA. In the published clinical series already mentioned above and in the protocol of the phase III MISTIE trial the drain was closed for 1 hour after rtPA application. Then the drain was opened for passive flow by gravity [9–11, 14, 15, 18–20]. Considering this clinical practice we assumed that the fibrinolytic effect, which can be reached by a single rtPA dose in 1 hour, was determined as 100%. Assuming this, in our in vitro series the lysis rates after 5 min exposure to rtPA were $53.22 \pm 3.9\%$, after 15 min $79.41 \pm 1.7\%$, and after 30 min $85.38 \pm 1.5\%$ [21, 22]. These results correspond well to the half-life of rtPA, which is about 6 min [29]. But it raises the question, whether it is necessary to close drains for 1 hour, if app. 80% of the lysis can be achieved within 15 min [21, 22]. Translating these findings to the clinical situation with a faster opening of the drain could lower time of increased intracranial pressure and increase the effectiveness of hematoma evacuation.

The question of rtPA efficacy in older hematomas is still a matter of debate. The phase III MISTIE protocol excludes patients with symptoms more than 24 h prior to the initial diagnostic CT scan and surgery should be intended in 72

hours after ictus [20]. In our in vitro series we found a significant higher lysis rate in newly formed clots of 1.5 h compared to the 24 h and 48 h old clots [21, 22]. There was no difference in the lysis rate of 24 h and 48 old clots, suggesting that the relevant changes causing rtPA resistance take place within the first 24 h. Furthermore, repetitive rtPA administrations did not result in a linear decrease of clot volume. The largest volume reduction occurred after the first 2 rtPA applications.

5. Limitation and Advantages of This Model

These results, however, have to be interpreted with caution. This in vitro model does not consider the perifocal environment of the brain tissue surrounding the intracerebral hemorrhage. Many inhibiting and activating factors may influence the maturation of clots but also the activity of the administered drugs. Large animal models may be superior to our in vitro model in assessing this question.

The advantages of this model are its reproducibility and reliability of clot size and structure and the usefulness in numerous future experiments, focusing not only on rtPA kinetics. Effects and kinetics of several other lytic drugs and their combination may be rapidly compared and investigated in this model. Furthermore the lytic activity of different ultrasound modes and physics can be assessed easily in this in vitro model. The model offers the perspective of assessing an individualized fibrinolytic therapy using lytic drugs alone, sonothrombolysis alone, or a combination of lytic drugs and sonothrombolysis. Therapeutic issues concerning clot age and coagulation status are important, which can be easily screened in such a model system before testing in an animal model and finally in the patient.

6. Conclusion

We established an easy and robust in vitro model of ICH, which allows a high number of repetitive experiments under controlled conditions. We applied this model to assess the optimal dose and timing of rtPA lysis in human blood clots and found a surprisingly low optimal dose of only 1 mg rtPA independent of the clot size (25, 50 mL, resp.). Further we showed that 80% of the lysis occurs within the first 15 min of incubation. The data suggests that current protocols for rtPA based ICH therapy could possibly be optimized by using smaller doses and shorter incubation times. This might in the future allow a faster reduction of intracranial pressure than achieved in current clinical protocols.

Ethical Approval

This study was approved by the local Ethical Committee of Rhineland palatinate.

Consent

All blood samples were taken after informed consent of healthy volunteers.

Disclosure

Results of this article are parts of the ongoing doctoral thesis of Hendrik Müller-Werkmeister.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Naureen Keric and Julia Masomi-Bornwasser equally contributed to this work.

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Review Article

Hydrocephalus after Subarachnoid Hemorrhage: Pathophysiology, Diagnosis, and Treatment

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Hydrocephalus (HCP) is a common complication in patients with subarachnoid hemorrhage. In this review, we summarize the advanced research on HCP and discuss the understanding of the molecular originators of HCP and the development of diagnoses and remedies of HCP after SAH. It has been reported that inflammation, apoptosis, autophagy, and oxidative stress are the important causes of HCP, and well-known molecules including transforming growth factor, matrix metalloproteinases, and iron terminally lead to fibrosis and blockage of HCP. Potential medicines for HCP are still in preclinical status, and surgery is the most prevalent and efficient therapy, despite respective risks of different surgical methods, including lamina terminalis fenestration, ventricle-peritoneal shunting, and lumbar-peritoneal shunting. HCP remains an ailment that cannot be ignored and even with various solutions the medical community is still trying to understand and settle why and how it develops and accordingly improve the prognosis of these patients with HCP.

1. Introduction

Hydrocephalus (HCP) is a serious and common complication in the clinical course of subarachnoid hemorrhage (SAH), which continues to be vague until now. According to various background and clinical circumstances, wide range of incidence of HCP in SAH patients from 6 to 67% has been reported; in most recent studies this percentage is about 20% ~30%.

HCP occurs in about one fifth of patients in the early course (acute in the first 3 days or subacute in the 4–14 days) of SAH, while chronic hydrocephalus happens in 10%–20% of patients later in the course of SAH (after 2 weeks). Regardless of the occurring period, HCP impairs patient's neurologic function and leads to deterioration of functional outcomes, especially with intraventricular hemorrhage (IVH), even if the primary SAH has been treated [1]. On the contrary, better outcomes occur if SAH is recognized early and treated.

Despite not having satisfactory preventive treatments, there have been several therapeutic methods developed to deal with hydrocephalus or to minimize the necessity of permanent shunts. Intraoperatively, lamina terminalis fenestration (LTF) with thorough lavage of blood clots out of ventricles and cisterns is carried out in order to reconstruct the normal flow course of cerebrospinal fluid (CSF) and also to eliminate the impairments by blood clots and its by-products. Postoperatively, temporary intraventricular or lumbar drainage is a technique used to transfer CSF reabsorption. For patients without intraventricular catheters or lumbar drains but with persistent symptoms, serial lumbar punctures are necessary. Despite these efforts to prevent the occurrence, a considerable number of patients are in need of a perennal shunt for CSF.

In this review we summarize the research of SAH-induced HCP and discuss the etiology, diagnosis, and treatment. With this field advancing thanks to the efforts

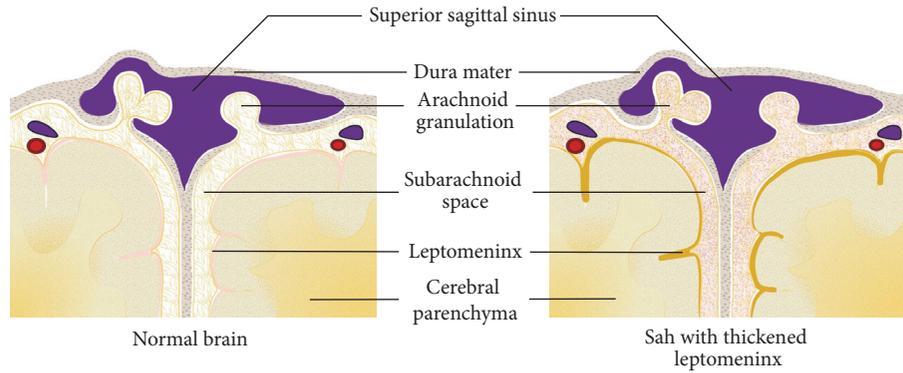


FIGURE 1: After SAH, the subarachnoid space is filled with blood cells and products. Leptomeninges is detected thickened with hemosiderin deposits, which has also been confirmed histologically.

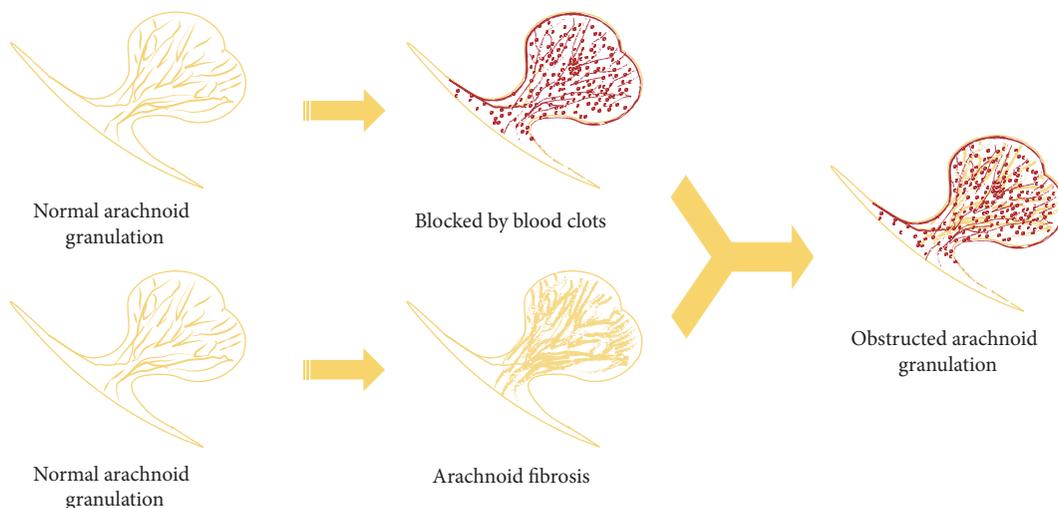


FIGURE 2: This picture shows the major pathological mechanisms in arachnoid granulations; the upper ones demonstrate the blood clots and corresponding products blocking the outflow tract of CSF and the inferior ones show the fibrosis of arachnoid membrane meanwhile.

of many researchers, questions and problems on treatment and prevention remain to be solved and applied to clinical practice.

2. Etiology

About one third of patients admitted with SAH have permanent CSF diversion. A large-scale meta-analysis reported that shunt-dependent HCP accounts for a proportion of 17.4% [2]. Patients with acute course, in-hospital complications, IVH, poor admission status, rehemorrhage, location of ruptured aneurysm, and age ≥ 60 reported a higher risk of shunt dependency [3–5].

Achievements and progress in studying hydrocephaly inevitably fall short of elucidating the entire mechanism of HCP after SAH. The theories mentioned hereinbefore meet the questions of researchers approximately through damage to arachnoid granulations (AGs) as well as to brain tissue. Mechanisms seem to be interweaving among the pathogenesis of acute and chronic HCP. It is generally accepted that the inflammatory reaction (either chronic or acute) and the

ensuing fibrosis process impede fluent CSF flow outward to sinus, terminally from AGs. Beside the proliferation of leptomeningeal cells (Figure 1), studies at present primarily target the pathological obstruction of AGs, including the mechanical blockage and fibrosis of AGs (Figure 2). Researchers have been long working on attenuating this pathogenesis to deal with HCP [6, 7].

Researchers mostly focus on the pathophysiology of brain injury after SAH, and prevalent theories include inflammation, apoptosis, autophagy, and oxidative stress (Figure 3). Vasospasm of choroidal artery probably originates HCP through stenosing the aqueduct and impairing ependymal cells after SAH [8]. Devascularization of brain parenchyma likely results from sequential vasospasm of SAH and is confirmed to induce the proliferation of neural stem cells directed by glia cells [9]. Gliocytes, different from other organs of the body, play the destructive and curative roles and release plenty of cytokines when the brain suffers various lesions [10]. Matrix metalloproteinases are believed to be crucial and versatile participants in breaking down blood-brain barrier (BBB) [11], and the tissue inhibitors of matrix

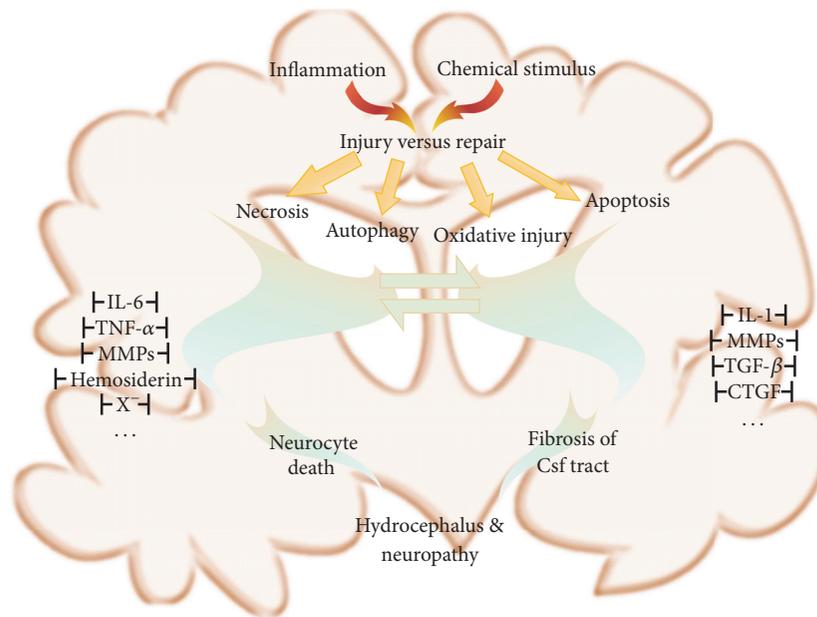


FIGURE 3: This picture shows some broadly verified molecules or pathways that are involved in the pathophysiology of hydrocephalus caused by SAH.

metalloproteinases have been verified to share the homologous protective effects in vasospasm after SAH for BBB integrity in apoplectic patients [12]. In addition, researchers found that the vegetative nervous system plays an auxiliary role in the inflammatory response and may contribute to the breakdown of BBB, which consists of glia cell both structurally and functionally [13]. Vascular endothelial growth factor protein levels rise and restrict the growth of abnormal blood vessels [14]. Subsequently, the hypersecretion of CSF triggers or exacerbates its circulatory disorder and eventually leads to HCP.

Acute HCP contributes to the causes of early brain injuries [15], usually thought as the noncommunicating (or obstructive) type, and is largely attributed to the mass effect or blood clots within the ventricles and aqueduct, preventing CSF flow out of the cranial vault. In addition, inflammation is believed to be the crucial biomolecular mechanism that induces acute HCP through disruption of BBB [16]. Nevertheless, recent research illustrated radiologically similar performances between acute and chronic HCP, indicating partially similar pathogenesis. Phase-contrast MRI demonstrated that chronic HCP turns out to be of communicating form; however, some of these individuals still develop acute HCP after SAH despite the absence of IVH or blood clot in the ventricles [17, 18]. Parallel parameters of CSF flow found in their studies also indicated that obstruction might not be sole initiator of acute HCP. Additionally, Kanat et al. postulated that blood clots play the initial role in triggering hypersecretion of CSF and fibrosis of arachnoid granulations, leading to long-term communicating HCP rather than merely aqueduct obstruction or stenosis [19]. Whether it is communicating, obstructive, or a pathophysiological hybrid, it may directly affect the treatment decision and corresponding prognosis of these patients. Despite many discoveries and advances, more

evidence is needed to uncover and explain the etiology of acute HCP following hemorrhage.

Conversely, a considerable number of patients with chronic HCP have no increased intracranial pressure (ICP) and with abundant evidence emerging in the pathway of fibrosis, there is a general consensus that chronic HCP is of “communicating” type, attributed to the fibrosis and adhesions of the leptomeningeal and arachnoid granulations. Blood products and transforming growth factor have long been postulated to play important roles in the pathophysiological processes after SAH, including chronic HCP. Intraventricularly injected iron (ferrous chloride or ferric chloride) or lysed red blood cells can similarly lead to HCP in rats [20]. In addition, Strahle et al. also detected cell deaths in neonatal rat model through pathological sections [21], which has testified the very critical effects in the mechanisms. Furthermore, necrosis of brain cells and disruption of BBB induced by iron are also depicted in rats [22], which makes this postulation more eloquent. Given all the previous research, preclinical research is supportive of the idea that oxidation accounts for the precise mechanisms of pathogenesis induced by iron [23], initially termed “ferroptosis” [24]. But more evidence is needed to further unravel the proceedings and connections between “ferroptosis” and HCP, and we are longing for a convincing clinical trial to testify whether removing the blood clot or subarachnoid blood lavage in the initial stage of SAH will have a definite positive outcome in these patients.

3. Diagnosis

Compared with detection of chronic HCP occurring during or after the course of SAH, it is more difficult to clinically diagnose acute HCP, which can be misleading or concealed by SAH accompanied with headache, nausea, or conscious

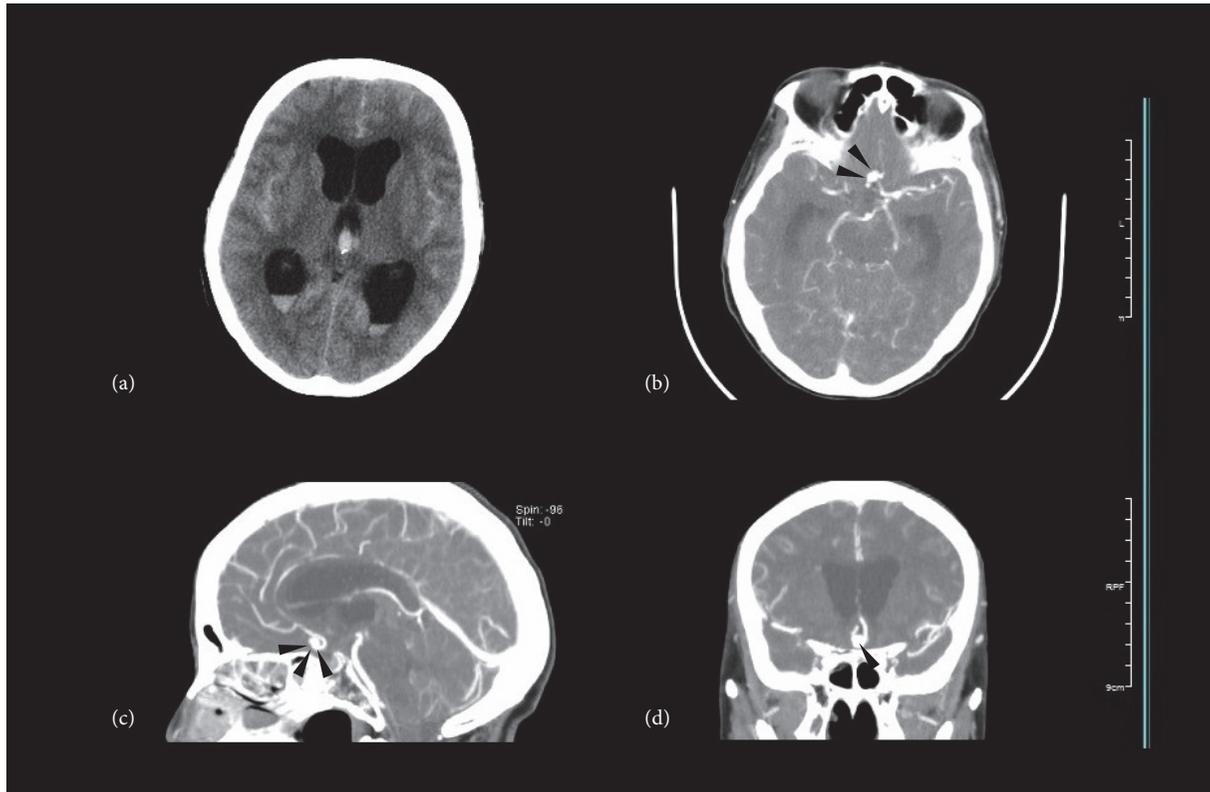


FIGURE 4: This picture shows a case of acute HCP induced by aneurysmal SAH, typically with an IVH. It happened as soon as the occurrence of SAH. (a) The CT scans above show widely hemorrhagic sulci and arachnoid cisterns with dilated lateral and third ventricles containing blood. (b), (c), and (d) Immediate CTA after admission locates the culprit aneurysm on the anterior communicating artery (marked by black arrows).

disturbance. Since it involves ventricular dilation anatomically, its recognition is primarily based on radiographic techniques, especially CT scans (Figure 4). The bicaudate index (BCI) and relative bicaudate index (RBCI) (calculated, resp., in different age groups) have been commonly accepted and widely applied as the diagnostic measurements since the study of Gijn and colleagues in 1980s (as shown in Figure 5) [17, 25–28]. And peers draw a conclusion that if not detected promptly before $RBCI > 1.6$, the effort to launch a drainage surgery could be in vain because of unimproved outcomes [29]. Still, the form and shape of dilated ventricles in patients differ a lot, and the authors suppose it is more accurate to measure the volume of ventricles and calculate the dilation rate [30].

Advances in radiological imaging and studies and useful methods such as diffusion tensor image (DTI) [31] and diffusional kurtosis image (DKI) are utilized [32], but CT is still the fastest and most efficient diagnostic one for HCP. Moreover, MRI gives much more details regarding whether or not and how brain parenchyma is damaged by ventricular dilation. What is more, we can observe precisely the morphology of the aqueduct and dynamics of CSF and subsequently know if it is blocked or stenosed [17, 18]. These advanced examinations provide more details in patients than CT scans, which are likely to facilitate unveiling the etiology

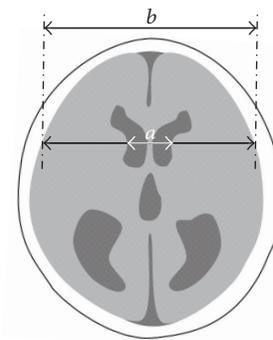


FIGURE 5: This picture simulates how to calculate the BCI, namely, the severity of HCP, the ratio. Segment “a” is the distance between caudate nuclei and “b” is at the same level the width of brain. The ratio “a/b” of respective group of age, that is, relative bilateral caudate index is also widely accepted among researchers.

and pathogenesis of HCP. One study demonstrated both the altered microstructure and water molecule movement within neural axons and intra- or extracellular space in patients with idiopathic normal pressure hydrocephalus (iNPH) by DTI and DKI [33]. These findings may be useful in evaluating the brain damage after SAH and HCP [34].

TABLE 1: Comparison among dominant treatment methods.

	Lamina terminalis fenestration (LTF)	Ventricle-peritoneal shunting (VPS)	Lumbar-peritoneal shunting (LPS)
Advantages	(1) Less injuries; (2) no implanted materials and less related complications; (3) conform to normal CSF dynamics; (4) milder fluctuation of ICP	(1) Higher availability; (2) more beneficial outcome	
Indication	Preferred for obstructive HCP, especially for those with mesencephalic aqueduct obstructed	Communicating HCP; some obstructive patients	Only for communicating HCP \geq 2-year-old
Common complications	CSF leakage, meningitis, bleeding, basal artery injury, hypothalamic damage, epilepsy	Device fault, infection, excessive shunt, intracranial hypotension, slit ventricles, subdural hematoma or hydrocephalus, displacement, visceral injury, epilepsy	

4. Predictive Factors

A considerable number of patients are exposed to the risk of shunt-dependent HCP after SAH. Earlier diversion of CSF results in less damage to brain parenchyma. Difficulties exist in deciding whether to intermittently launch drainage or perform surgery to divert CSF secreted beyond absorption. It is important and beneficial to predict shunt-dependence beyond its clinical performances [35]. Patients with acute course of HCP, in-hospital complications, IVH, high Hunt and Hess Scale score (or low initial Glasgow Coma Scale or high Fisher score), rehemorrhage, posterior circulation location of ruptured aneurysm, and age \geq 60 have been reported to be at a higher risk of shunt-dependency [3–5]. Other research reported similarly higher risk of HCP with posterior circulation aneurysm, IVH, greater hemorrhage volume, and older age [4, 5, 28, 36]. Dependency on factors like economy, medical development, and methods to cope with ruptured aneurysms also leads to deferent incidences of shunt-dependent HCP [5].

In addition, some researchers attempt to find a precise and measurable way to foresee the perennial shunting necessity. In the study of Hoh et al., symptomatic aneurysms are found likely larger and more likely to cause obstructive hydrocephalus, which may need a drainage operation [37]. Yamada et al. in 2012 introduced a discriminant function relevant to determining the need for VPS after SAH [38]. The sensitivity and specificity were at 85.3% and 87.2%, respectively, which are high enough for predicting shunt-independence. This is favorable to earlier surgical performance and prevents damage caused by ventriculomegaly. More evidence and cases are needed to develop a function model more clinically applicable and usable.

5. Treatment

5.1. Medical Treatments. Common medical treatments for HCP mainly include acetazolamide and mannitol. It has been testified by perennial clinical practice that medication does not reduce the possibility of subsequent surgical drainage, with extra side effects. It is now applied in hopes of putting off shunt-placement surgery and preoperative preparation.

Along with the gradual disclosure of mechanisms in HCP in recent years, some experimental agents are found to be potentially effective in improving the outcomes of patients [7, 39]. Minocycline is reported to be effective in reducing the gliosis and delaying the development of HCP in rat model [40]. And decorin may be beneficial for the long-term of HCP [7]. On the other hand, in neonatal rats with germinal matrix hemorrhage, deferoxamine attenuates long-term complications including posthemorrhagic dilation of ventricles [41].

SAH shares similar mechanisms with intracerebral hemorrhage and contributes to detrimental processes that include HCP and brain apoptosis. In this regard, they might have similar treatments. Trichostatin A (TSA), histone deacetylase inhibitor which enhances autophagy, contributes to alleviation of neuronal apoptosis, improvement of neurological function, and attenuation of brain injury following SAH [42], potentially leading to slighter fibrosis of meninx and better outcomes of patients with HCP.

5.2. Surgical Treatments. Despite a considerably high incidence of complications, about 50%, shunt failures within 1 year, about 30%, and a number of patients in need of a secondary surgery to revise the catheter, surgery is still the preferred treatment for HCP. The aim of surgical treatment is to improve the neurofunction by CSF flow diversion rather than restore the original cerebral structure. Surgical protocol differs depending on the type of hydrocephalic lesion and the conditions of individual patients. The optimal time for surgical treatments remains controversial. Three predominant surgical methods for HCP are compared with each other in Table 1.

5.2.1. Lamina Terminalis Fenestration (LTF). Reported to have less complications and being favorable in reducing shunt-dependent occurrence [43–45], surgeons incline to launch LTF during surgical operation for acute SAH after lavage of blood clots in the subarachnoid space to avoid posthemorrhagic obstruction of CSF flow. However, some other researchers questioned the efficacy of LTF to cut down shunt-dependence of patients [46]. As mentioned in our passage about the pathogenesis of acute HCP, LTF does

not terminate or delay the fibrotic process of leptomeninges and arachnoid granulations, hence possibly improving CSF dynamics. Authors remain suspicious of its effects and long-term outcomes, mainly the shunt-dependent incidence on acute patients, especially those who suffer from communicating HCP without early diagnostic evidence.

5.2.2. Ventricle-Peritoneal Shunting (VPS). VPS is currently the most widely applied surgical method to deal with HCP. According to a systematic review involving 41,789 patients with aneurysmal SAH in 66 published studies, the overall VPS insertion rate was 12.7% [47]; 31.2% patients required a VPS for acute HCP after aneurysmal SAH, regardless of whether it was after endovascular or surgical treatment [48].

However, even though it is the most commonly applicable surgical protocol, VPS bears an inevitable high risk of complications and failures. A 10-year follow-up among 14,455 individuals who underwent VPS showed 32% had cumulative complications at 5 years [49]. Another clinical study exhibited 51.9% patients accepting VPS requiring shunt revision(s) [50]. Occurrence of complications mostly attributes to the implantation of the catheter and communication between ventricles, cisterns, and enterocoelia. The way in which surgeons implant the tube and how they set the parameters of the CSF sluice play a significant role in determining the outcomes of patients.

5.2.3. Lumbar-Peritoneal Shunting (LPS). LPS is usually performed as a supplementary solution for patients who suffer from communicating HCP that are not suitable for VPS. Compared with VPS, LPS involves a much shorter catheter, consequently slighter complications such as excessive shunt, intracranial pressure fluctuation, slit ventricles, and infection. On the other hand, LPS occupies a more narrow scope of application for curing HCP.

6. Conclusion

HCP occurrence after SAH presents with various clinical characteristics and mysterious biomolecular mechanisms that are still not addressed. Even though some studies demonstrated the pathophysiology includes fibrosis and obstruction of arachnoid, corresponding risk factors, which are generalized by predecessors, still contribute limitedly to avoiding HCP. Several surgical methods including LTF, VPS, and LPS are available but deficient in avoiding or treating hydrocephalus. However, the medical research community continues to discover mechanisms involved and more efficient and beneficial treatments for patients.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

Dr. Sheng Chen and Dr. Jinqi Luo contributed equally to the paper.

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Clinical Study

Medical and Interventional Therapy for Spontaneous Vertebral Artery Dissection in the Craniocervical Segment

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Background and Purpose. Spontaneous vertebral artery dissection (SVAD) is an important reason for posterior-circulation-ischemic stroke in the young and middle-aged population. Although some previous reports reveal a favorable outcome with conservative therapy, it is still controversial in the treatment of SVAD in some specific patients. Herein, we present our 10 years of clinical experience for SVAD at this location. **Material and Methods.** 20 patients with 20 SVADs in V2 and V3 segments were retrospectively studied. Clinical manifestations and imageology materials were collected and analyzed. All the patients underwent anticoagulation except for one patient because of contraindication. 14 patients underwent Wingspan stents implantation with general anesthesia. **Results.** In our sample, ischemia (infarction or transient ischemic attack, TIA) was found in all the patients. Angiographic stenosis and dissection aneurysm were the most common findings in the segments mentioned above. 19 of the patients (95%) got the excellent imageological and clinical outcomes. **Conclusions.** According to our experience in this group, although anticoagulation is effective in vertebral artery dissection, interventional therapy for SVADs in V2 and/or V3 segments is preferred in some specific patients. Stent with higher radial supporting and flexibility, such as Wingspan stent, is suggested.

1. Introduction

Spontaneous vertebral artery dissection (SVAD) is a rare condition that occurs in the young and middle-aged population with an estimated annual incidence between 1 and 1.5 per 100,000 [1, 2]. The typical symptoms are ischemia and/or subarachnoid hemorrhage (SAH), which were usually seen in the extracranial and intracranial segment, respectively [3, 4]. SVAD is most commonly observed in the V2 and V3 segments of the vertebral artery (VA) [5, 6]. Although connective tissue abnormalities, hyperhomocysteinemia, fibromuscular dysplasia, and hypertension are factors associated with SVAD, the tortuous and a relative large range of motion of VA at craniocervical level are perhaps the major predisposing factors. As the greatest risk of stroke in craniocervical dissections appears to occur in the first few weeks [7], it is very important to treat these patients with effective methods in time. Although medical therapy has shown a favorable outcome, there are still more than 10% of the patients with

serious disability and even death [8]. Until now, few studies involve in clinical relapse and imageological characteristics. Despite the lack of standard guidelines for the treatment of SVADs in V2 and V3 segments, it is the common practice to treat aggressively patients who present with repeated relapse of ischemia during conservative treatment with maximum of anticoagulation. The purpose of this study was to describe the characteristics of clinic and imageology in these specific patients and identify the safety and efficacy of Wingspan stent in the treatment of SVAD in V2 and V3 segments as an alternative method.

2. Materials and Methods

20 patients with SVADs in the V2 and V3 segments confirmed by digital subtract angiography (DSA) were retrospectively analyzed between May 2004 and April 2014 at our center. There was only one patient diagnosed as fibromuscular dysplasia presenting with the stenosis of left carotid artery and

TABLE 1: Summary of characteristics of the 14 patients with SVAD treated by stenting.

Patient	Sex	Age	HT	FHS	DM	Signs/symptoms	SVAD location	VA dominance	Devel of con-VA	NIHSS	mRS	Follow-up (months)
1	m	39	-	+	-	A/N/T/V/H	V3(L)/DoVA(-)	+	+	2	0	6
2	m	38	+	-	-	A/N/V/P	V3(R)/DoVA(+)	+	-	1	0	12
3	m	41	-	-	-	N/T/V/P	V2(L)/DoVA(+)	+	+	1	0	9
4	m	29	-	-	+	A/N/V	V2(R)/DoVA(+)	+	-	1	0	36
5	m	46	-	-	-	A/N/T/V	V3(R)/DoVA(+)	+	-	1	0	24
6	m	38	-	-	-	A/N/V/P	V3(L)/DoVA(+)	+	+	1	0	36
7	m	45	+	-	-	A/N/T/H	V2(L)/DoVA(+)	+	-	1	0	60
8	f	33	-	-	-	D&D	V3(L)/DoVA(+)	+	-	3	1	48
9	m	37	-	-	-	A/N/T/V	V3(R)/DoVA(+)	+	+	1	0	48
10	m	40	-	-	-	A/N/P	V2(L)/DocVA(-)	+	+	1	0	30
11	f	46	+	-	-	A/N/T/H	V3(R)/DoVA(+)	+	-	1	0	24
12	m	28	-	-	-	A/T/H	V2(L)/DoVA(+)	+	-	1	0	30
13	m	35	-	-	+	A/N/T/H	V3(L)/DoVA(+)	+	+	1	0	36
14	m	36	-	-	-	A/V/H	V2(L)/DoVA(+)	+	-	1	0	48

A: ataxia; N: nystagmus; T: tinnitus; V: vomiting; H: headache; P: neck pain; D&D: dysphagia and dysarthria; m: male; f: female; SVAD: spontaneous vertebral artery dissection; VA: vertebral artery; Devel of con-VA: development of contralateral vertebral artery distal to the orifice of PICA (posterior inferior cerebellar artery); NIHSS: National Institute of Health stroke scale; mRS: modified Rankin score; V3: the third segment of vertebral artery; V2: the second segment of vertebral artery; L: left; R: right; DoVA(+): dissection on the dominant vertebral artery; DoVA(-): dissection on the nondominant vertebral artery; HT: hypertension; FHS: family history of stroke; DM: diabetes mellitus.

dissection of left VA, who was treated conservatively, because the medical therapy was effective. And the six patients with hypertension belonged to the two groups (conservative and interventional groups) equally. There were no histories of other risk factors, such as connective tissue abnormalities and hyperhomocysteinemia. Pre- and postoperative neuroimaging were studied (including magnetic resonance angiography, MRA, DSA, and/or magnetic resonance imaging, MRI). Patients with SVADs in the segment of V1 and V4 (intracranial segment) were excluded. The diagnosed standards of SVADs on DSA included (1) irregular stenosis of VA; (2) double lumens; (3) intimal flap; (4) string sign; (5) string beads sign; (6) aneurysmal dilation; (7) all these patients who did not suffer from trauma or iatrogenic injury. All the 20 patients were followed up with DSA ranging from 6 to 60 months (mean term 28.6 months). The descriptive statistic methods (mean value, percentage, and standard deviation) were used in this study.

2.1. Patients' Characteristics and Imageology Findings. Computed tomography (CT) and MRI were performed in all the 20 patients (6 females, 14 males; mean age: 37.05 years, range: 23–46 years) presenting with acute ischemia in the posterior circulation (pons, cerebellum, and/or medulla), and DSA was used for following up. The associated signs and symptoms included cerebellar ataxia ($n = 12$), nystagmus ($n = 15$), dysphagia and dysarthria ($n = 1$), tinnitus ($n = 10$), vomiting ($n = 8$), neck pain ($n = 6$), and headache in ipsilateral occiput ($n = 8$). Clinical grading and outcome of following up (6 months after the initial treatment) were evaluated by National Institute of Health stroke scale (NIHSS) and modified Rankin

score (mRS), respectively. The patients' characteristics, dissection location, vertebral artery dominance, and imageology findings were listed on Tables 1 and 2.

2.2. Medical and Interventional Therapy. Six of the patients underwent a usual treatment with intravenous heparin followed by oral warfarin with a target international normalized ratio (INR) of 2.5 (range, 2.0–3.0) for 3 months. Although under the maximal dosage of anticoagulation with INR of 3.0 for 14 days, 13 of the patients underwent stents implantation with the preparation of double-antiplatelets (300 mg aspirin and 75 mg clopidogrel oral daily) as an alternative because of the relapse of stroke and the persistent positive signs and symptoms (the specific evaluation criteria and definition of "the relapse of stroke and the persistent positive signs and symptoms" in this study referred to symptomatic patients who suffered from SVADs with the treatment of medicine for at least two weeks, and the positive signs (cerebellar ataxia, nystagmus, dysphagia, and dysarthria,) and symptoms (tinnitus, neck pain, and headache) were persistently existing or even worse, or other neural dysfunction appeared). One of the patients did not receive anticoagulation for the multiple and extensive infarcts in the cerebellum and occipital lobe followed by a secondary SAH and ventricle hemorrhage. External ventricular drainage was performed in this patient for obstructive hydrocephalus and then followed by stenting 4 weeks later (the eclectic dosages of aspirin (100 mg) and clopidogrel (25 mg) were adopted 3 days before procedure and maintained for 3 months, and then the clopidogrel was stopped, and the aspirin was still going on. During the first week after stenting and the 3 days before procedure, we

TABLE 2: Summary of characteristics of the 6 patients with SVAD treated conservatively.

Patient	Sex	Age	HT	FHS	DM	Signs/symptoms	SVAD location	VA dominance	Devel of con-VA	NIHSS	mRS	Follow-up (months)
1	f	37	-	-	-	N/H	V3(L)/DoVA(-)	+	+	0	0	30
2	m	39	+	-	-	N	V2(R)	-	+	0	0	24
3	f	46	+	-	-	N/T	V2(L)	-	+	0	0	36
4	f	27	-	-	-	P/H	V2(R)	-	+	0	0	12
5	f	23	-	-	-	N/T	V2(R)	-	+	0	0	11
6	m	38	+	+	-	P	V2(L)	-	+	0	0	12

N: nystagmus; T: tinnitus; H: headache; P: neck pain; m: male; f: female; SVAD: spontaneous vertebral artery dissection; VA: vertebral artery; Devel of con-VA: development of contralateral vertebral artery distal to the orifice of PICA (posterior inferior cerebellar artery); NIHSS: National Institute of Health stroke scale; mRS: modified Rankin score; V3: the third segment of vertebral artery; V2: the second segment of vertebral artery; L: left; R: right; DoVA(-): dissection on the nondominant vertebral artery; HT: hypertension; FHS: family history of stroke; DM: diabetes mellitus.

monitored the coagulating and platelet functions consecutively). For the patients with stenting, double-antiplatelets were needed at least 3 days before and 3 months after the procedures, then with a 100 mg oral aspirin per day. On the day of procedure, a bolus of 2500 U of heparin was administered. During the duration of procedure, drip infusion through the 6 F-guiding catheter of 1000 U of heparin was administered continuously. Activated clotting time was controlled between 250 and 300 seconds. Stent implantations, under general anesthesia, were performed on a biplane angiographic unit (LCN+, GE company, US). SL-10 microcatheter (Stryker, US) was used to facilitate the guider wire to go across the lesion of the involved VA. The gateway balloon and Wingspan self-expandable stent (Stryker, US) were chosen according the artery diameter and length of the involved artery. For the treatment in subacute phase, predilatation with gateway balloon was preferred in order to diminish the residual stenosis and get an anatomical healing.

3. Results

In our sample, men were more frequently affected than women (14:6), and the mean age was 37.05 years. Ischemia (infarction or transient ischemic attack, TIA) was found in all the patients (infarction, 17; TIA, 3). Subarachnoid and ventricle hemorrhage secondary to the infarction were seen in one. Eleven dissections were found in the V2 segments and 9 in V3. Angiographic stenosis (12/20) and dissection aneurysm (6/20) were the most common findings in the segments of V2 and V3, and the last was intimal flap (2/20). Unilateral VA dominance was found in 15 patients and VA equability in the others. Twelve lesions were on the dominant VA and three in the nondominant. Reangiography showed that two patients had extension of the dissections from V2 to V3 segment on the day of procedures. The average term of following up by angiography was 28.6 months (ranging from 6 to 60 months) in all of the patients. Nineteen of the patients (95%) got the excellent imageology and clinical outcomes (mRS = 0). The patient with subarachnoid and ventricle hemorrhage also had a good recovery (mRS = 1).

3.1. Technical and Clinical Results. Fourteen patients (six dissections in the V2 segment and eight in V3 initially),

twelve lesions on the dominant and two on the nondominant VA, underwent stent implantation successfully. Among the six dissections in the V2 segments, two were found to extend from V2 to V3 segment before stenting. There was no procedure related complication. Eighteen dissections were predilated through gateway balloon. Remnant stenosis (<10%, the initial stenosis rate was 80–95%) presented in one (7%). Thirteen of the fourteen patients got a satisfactory outcome without any residual stenosis and none of them presented with relapse of ischemia or positive symptoms and signs. The one with subarachnoid and ventricle hemorrhage also had a relative good recovery since the stent implantation. No case of hemorrhage was seen. Six to sixty months of following up showed that there was no in-stent stenosis, and the residual stenosis after the first stenting disappeared. None of them presented fresh infarct on MRI.

4. Discussion

This is the first study of SVAD in the craniocervical segment treated with medicine combined with self-expandable stent. Previous study has showed that the conservative therapy including anticoagulation and antiplatelet is effective for SVADs, especially for those in the extracranial segment [4, 5]. But according to the ten years of our experience about this kind of disease, it is not always the case.

In the samples of ours, fourteen patients (14/20) underwent stent implantations finally, because the anticoagulation or antiplatelet did not bring any efficacy. Although with a normative medical therapy, it was difficult to stop the relapse of stroke and eliminate the symptoms. So we had to use stents for these patients in order to recover the continuity of VA and diminish defluxion of thrombus. In the fourteen patients, there were twelve lesions on the dominant VA, and eight patients of them had no development of contralateral VA distal to the orifice of posterior inferior cerebellar artery (PICA). Stenosis caused by the dissection brought a catastrophic ischemia in the poster circulation without sufficient collateral compensation. Angiography of the left two patients with lesions on the nondominant VA showed the dissection-pattern and anatomy characteristics, which was associated with cerebellar infarction (Figures 1 and 2).

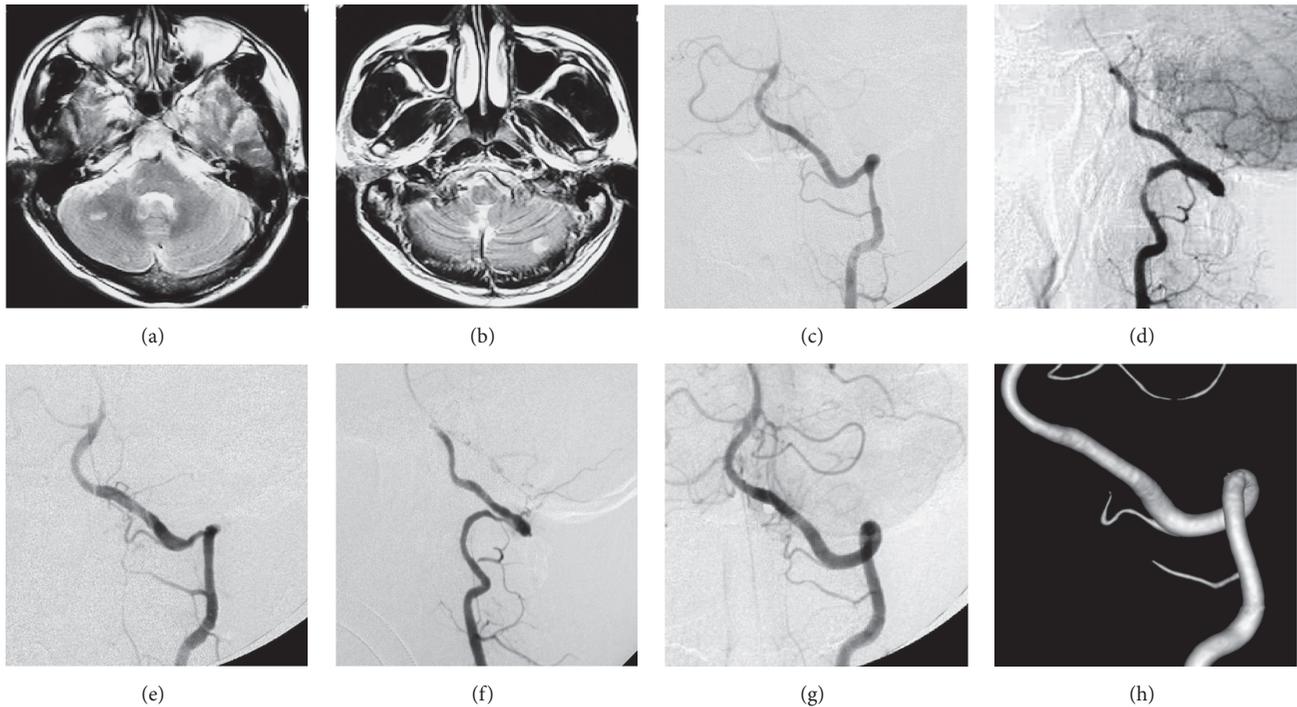


FIGURE 1: MRI ((a) and (b)) showed multiple infarcts in the bilateral cerebellum especially in the right cerebellar hemisphere. Frontal and lateral DSA ((c) and (d)) of left VA angiogram showed the dissection in the segment of V3. Stenosis was the major appearance. Although the left VA was the nondominant one, the embolus is easy to escape and enter into both the right and left AICA (more into the right one) due to neck motion and the characteristics of anatomy of right AICA (the VA distal to the dissection, inferior segment of BA, and the initial segment of right AICA are almost in a line). Frontal and lateral DSA showed the stenosis disappeared after stenting ((e) and (f)). 2-dimensional and 3-dimensional DSA ((g) and (h)) showed the anatomical healing of the involved VA when following up. MRI: magnetic resonance image; DSA: digital subtraction angiography; VA: vertebral artery; AICA: anterior inferior cerebellar artery; BA: basilar artery.

SVADs in the craniocervical segment are much more than those in the other locations. The certain reasons are not well known, but the relative extensive range of motion and tortuosity of VA has been thought as the usual cause. Hemodynamic and mechanical motion might be the reasons for the difficulty of dissection healing and even cause the dissection to extend forward into the distal part. In our samples, two patients with dissections in the segment of V2 were found to deteriorate on the angiography. The extensive dissection further aggravated the hemodynamic ischemia. This phenomenon, in some degree, might present an explanation for inefficacy of conservative treatment.

Previous study indicated that embolism was usually considered as the major reason (90%) for ischemia (stroke, TIA) [9–11], and the anticoagulation or antiplatelet therapy was effective for the majority of the patients. But in our group, only six patients (6/20) were treated conservatively and got the comparative good results. The left fourteen patients had to receive stent implantations because of continuous aggravation of the symptoms, even under a maximum of anticoagulation. The different outcome between our study and the previous was associated with asymmetry of VA and insufficient collateral compensation. For the fourteen patients with stenting, hemodynamic ischemia was the main reason in eight whose lesions were in the dominant VA with the contralateral nondevelopment of VA distal to the orifice of

PICA. Embolus escaping from the dissection was the other probable reason in the left six patients (two lesions in the nondominant VA and four in dominant VA).

The fact that hemodynamic ischemia and embolisms may occur in the same patients has to be kept in mind in clinical practice. When anticoagulation is not effective, stenting should be considered as an alternative method. In De Bray et al.'s prospective study of 22 consecutive vertebral artery dissections [12], about 50% of involved vertebral arteries were not found recanalized. For the young patients, to save the involved vessel is very important. When making the treatment strategy of SVAD, the dominance of VA, relationship between dissection and dominant VA, collaterals (patient's posterior circulation is supported by only a dissected vertebral artery without posterior communicating artery, and the contralateral vertebral artery development is poor), anatomy characteristics, and pattern of dissection should be analyzed adequately. Stenting as an alternative method should be suggested aggressively when the conservative therapy could not bring any further improvement. Fourteen days later, the endovascular therapy should be carried out if the conservative treatment does not work. Those who suffered from connective tissue abnormalities in progress were not suitable for stenting. When stenting is needed the Wingspan self-expandable stent is preferred as its characteristics of higher radial supporting and flexibility.

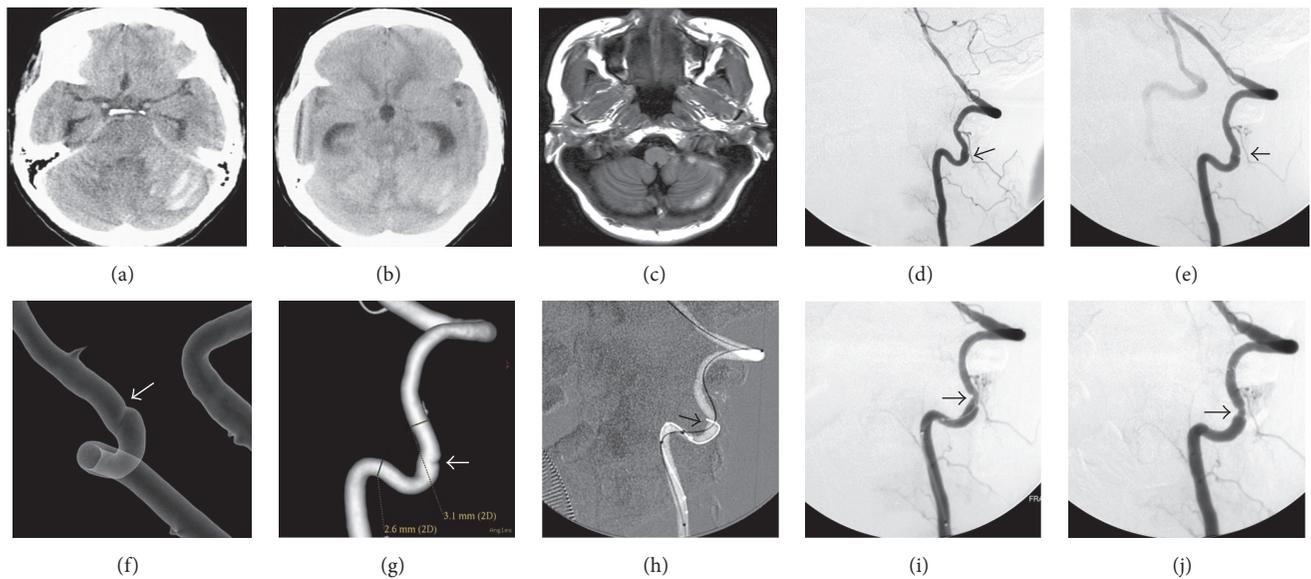


FIGURE 2: CT (a) showed lamellar infarct in the pons and worm of cerebellum and SAH. CT (b) showed obstructive hydrocephalus. MRI (c) showed SAH gradually decreased after ventriculoperitoneal shunt. Lateral and oblique DSA ((d) and (e)) of left VA showed the dissection in the segment of V2. Transparency and volume rendering ((f) and (g)) showed the intimal flap. It is the major appearance. During the procedure, the micro-guide wire was difficult to pass through the true lumen until the microcatheter was used (h and i). Lateral DSA showed slight remnant stenosis after stenting (j). CT: computed tomography; MRI: magnetic resonance image; DSA: digital subtraction angiography; VA: vertebral artery.

Because of the rare incidence of this kind of lesion, single-center-study really has the shortcoming in sample size. We look forward to a further multi-center-study in the future.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Phase I and Phase II Therapies for Acute Ischemic Stroke: An Update on Currently Studied Drugs in Clinical Research

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Acute ischemic stroke is a devastating cause of death and disability, consequences of which depend on the time from ischemia onset to treatment, the affected brain region, and its size. The main targets of ischemic stroke therapy aim to restore tissue perfusion in the ischemic penumbra in order to decrease the total infarct area by maintaining blood flow. Advances in research of pathological process and pathways during acute ischemia have resulted in improvement of new treatment strategies apart from restoring perfusion. Additionally, limiting the injury severity by manipulating the molecular mechanisms during ischemia has become a promising approach, especially in animal research. The purpose of this article is to review completed and ongoing phases I and II trials for the treatment of acute ischemic stroke, reviewing studies on antithrombotic, thrombolytic, neuroprotective, and antineuroinflammatory drugs that may translate into more effective treatments.

1. Introduction

Almost 2 decades after the demonstration of a decrement and in some instance absence of disability and the consequent approval of r-tPA for treatment of acute ischemic stroke (AIS), a plethora of research has been performed to better understand not only the mechanisms involved in protecting against AIS but also the synergy that different drugs produce in AIS treatment. After many years, a growing number of ischemic stroke patients lack other treatment options. A number of phase I and phase II clinical trials designed to develop better strategies to treat AIS are currently in progress or completed.

The understanding of ischemic stroke pathophysiological mechanisms is expanding. Comprehensive research in drug development builds upon experimental ischemic stroke models to recognize the mechanisms that underlie cerebral

ischemic injury. The lack of oxygen results in energy deprivation and the ischemic cascade starts with an arterial thromboembolic episode. The main aim of phases I and II clinical trials in AIS is to rescue and restore the ischemic penumbra within a specified therapeutic window. Otherwise, the abrogation of energy weakens ion homeostasis and provokes a rise in the extracellular concentration of K^+ , along with a decline in extracellular concentrations of Na^+ and Cl^- . This anoxic depolarization triggers not only the formation of reactive oxygen species, glutamate release, and dysregulation in intracellular Ca^{2+} levels, but also mitochondrial membrane collapse and induction of neuroinflammation [1]. Expedient recanalization is mandatory to avoid an ischemic cascade that generates neuronal tissue infarction. There are drugs in development that aim at inducing possible neuroprotective factors and/or pathways, facilitating immediate reperfusion to alleviate the ischemic injury, blocking platelet

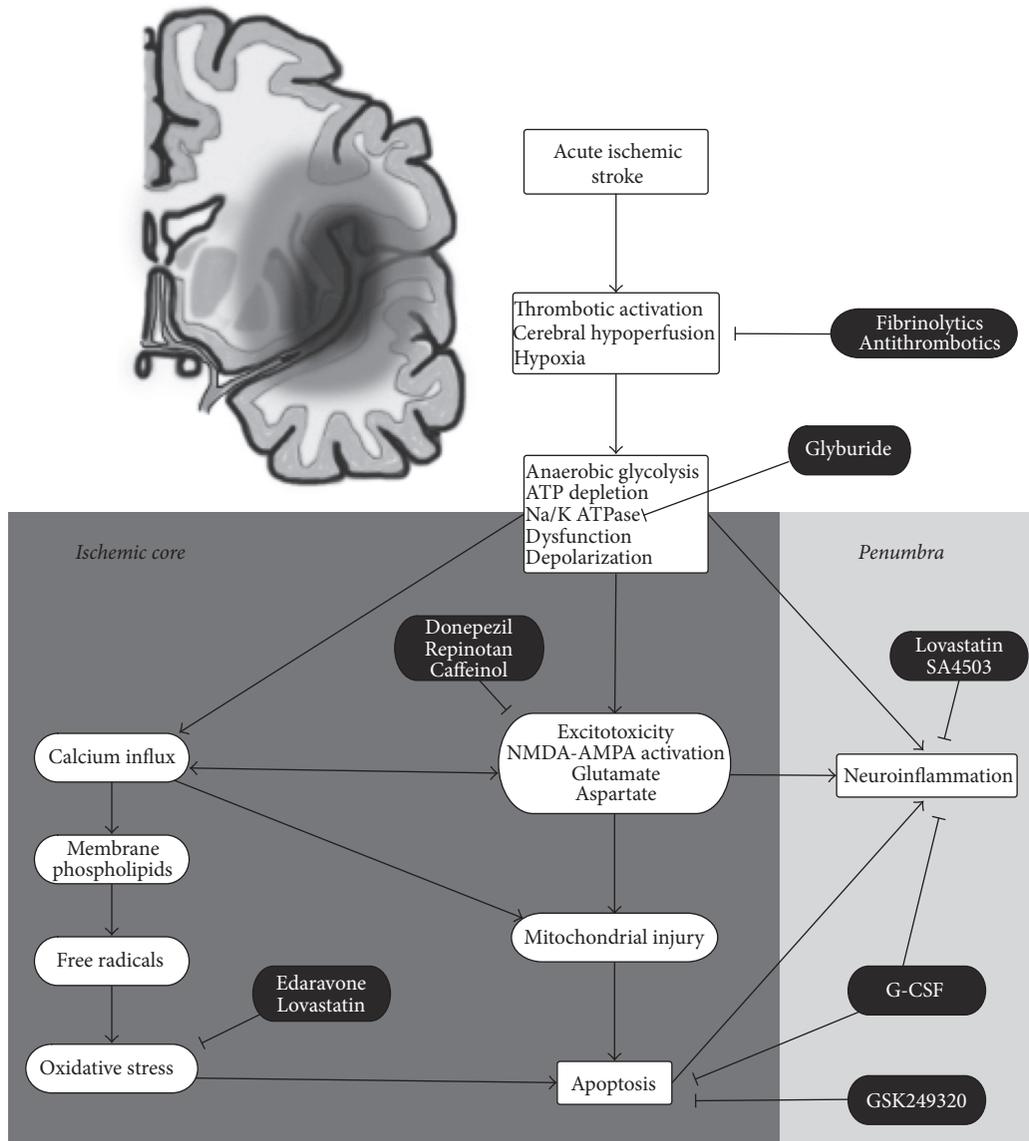


FIGURE 1: This figure provides an overview of the cellular mechanisms activated after cerebral ischemia and their respective targets by the different therapies (excitotoxicity and glutamate release, neuroinflammation with proinflammatory and anti-inflammatory states, etc.).

aggregation and coagulation, and degrading fibrin. Neuroprotective agents protect ischemic neurons in the acute phase of the ischemic stroke. We will provide an overview of the cellular mechanisms activated after cerebral ischemia and their respective targets in Figure 1.

2. Antithrombotic Drugs

2.1. Eptifibatide. Eptifibatide is an antiplatelet drug belonging to the glycoprotein IIb/IIIa inhibitor class. It binds to glycoprotein IIb/IIIa in between the IIb and IIIa arms of the activated platelet, effectively blocking the binding domain from fibrinogen, thus inhibiting thrombi formation. It has been studied in combination with aspirin, low molecular weight heparin (tinzaparin), and intravenous (i.v.) r-tPA therapy in several dosing regimens, since r-tPA alone is inadequate to

recanalize large arterial occlusions in approximately 50% of cases. In recent years, three trials have tried to establish safety of eptifibatide and they all kept their inclusion criteria constant [2].

The CLEAR trial was designed to establish the safety of eptifibatide in combination with r-tPA in the treatment for AIS. The endpoint was within 36 hours, finding that a dose escalation combination of reduced-dose r-tPA plus eptifibatide would justify further dose-ranging trials in AIS [3]. One group received low-dose r-tPA with eptifibatide (75 ug/kg bolus and 0.75 ug/kg/min infusion over 2 hours) and the other group received the standard dose of r-tPA. The primary safety endpoint was defined as the incidence of a symptomatic intracerebral hemorrhage (sICH) within the period of 36 hours. Even though this trial found promising results and was a randomized, blinded, safety trial measuring

low-dose r-tPA plus eptifibatide, it had a marked disparity in age and baseline NIH Stroke Scale/Score (NIHSS) between the combination therapy and the control groups [3]. Higher age and NIHSS are predictors of sICH after r-tPA treatment following AIS [4]. On the other hand, the study not only had the EuroQoL quality of life index and Stroke-Specific Quality of Life Scale administered at 3 months, but also has a 90-day outcome assessed by certified investigators in the NIHSS. Additionally, they received standardized training regarding the modified Rankin Scale (mRS), Barthel, Glasgow, and EuroQoL assessments. Furthermore, the study was not associated with acute treatment of patients, which brings more validity to the outcome score obtained. In light of these points, the CLEAR trial offered insights and hope for well-designed studies in the future in order to research the effects of this combination using a dose escalation regimen.

Following the CLEAR trial, investigators conducted the CLEAR-ER trial, a multicenter, double-blinded, randomized safety study that enrolled a total of 126 AIS patients (NIHSS score > 5) having mRS score as the primary efficacy outcome measurement. The CLEAR-ER trial added extra points to measure not only early patient improvement at 2, 24 hours, and 90 days per NINDS investigators' suggestion, but also systemic bleeding at 7 days after therapy. This trial consolidated the safety of combination therapy even though mild bleeding was found in the combination group (no interventions were necessary) and the sICH rate in the r-tPA group was greater than expected. A larger trial is necessary to address sICH differences from CLEAR-ER when compared to NINDS trials. However, combination treatment not only proved to be safe when given within 3 hours of symptom onset [5] but additionally proved to be realistic to pursue translation with combination of eptifibatide.

After the aforementioned trials were published, a full dose regimen trial was designed. CLEAR-FDR was a single-arm, prospective, open-label, multisite study using 0.9 mg/kg i.v. r-tPA within 3 hours of symptom onset followed by eptifibatide (135 μ g/kg bolus and 2-hour infusion at 0.75 μ g/kg per minute). A repeat NIHSS score was obtained at the end of the 2-hour eptifibatide infusion and at 24 (\pm 6) hours after r-tPA. 27 patients were enrolled in this trial, a number at least 3x less than the amount enrolled in the CLEAR trial and almost 5x less than the CLEAR-ER trials. Importantly the single-arm nonblinded study designs with enrollment by a single regional stroke team are also limiting factors [6].

Aside from the limitations of the CLEAR-FDR, there is a plan to investigate the dose response of r-tPA plus eptifibatide via a pooled analysis of all 3 completed trials before moving on to a phase III clinical trial. This will help estimate the variability of the trials. A demonstration of eptifibatide's mode of action is found in Figure 2.

2.2. Revacept. Revacept is a dimeric glycoprotein VI-fc that blocks glycoprotein VI-dependent pathways. By interfering with the vascular collagen site, it blocks vascular collagen in plaques or exposed by erosion thus reducing platelet adhesion. It was found to be safe in preclinical studies. Thirty healthy men received a single i.v. administration of 10, 20, 40, 80, or 160 mg revacept in a phase I study [7] that evaluated the

pharmacological parameters of the drug itself. Different from the CLEAR trials, its inclusion criteria were more selective to nonsmoking white men between the ages of 18–35, normotensive, and with body weight ranging from 75 to 85 kg. The concentration of revacept plasma was found to interrupt aggregation beginning 2 hours after the administration of the drug and produced significant inhibition 24 hours and 7 days following infusion with higher doses. Bleeding time was not significantly affected, and ADP (thrombin receptor activating peptide) dependent platelet aggregation was not changed. The drug's effect was longer lasting in humans than in previously conducted animal studies, which is likely due to the longer half-life. Among the pros and cons of the study, revacept was safe and well tolerated in a dose dependent pharmacologic profile.

Revacept is also being studied in conditions associated with stroke, such as carotid stenosis, that presents with microembolic signals (MES) [8]. MES are frequently found in patients with acute stroke. An ongoing phase II trial using a 20-minute single dose of revacept plus antiplatelet monotherapy (aspirin or clopidogrel) or monotherapy alone aims to reduce MES. Figure 2 demonstrates revacept action on decreasing platelet aggregation formation of a clot. Table 1 summarizes details of completed clinical trials testing these antithrombotic drugs.

3. Thrombolytic Drugs

3.1. Reteplase. Reteplase is a nonglycosylated deletion mutein of tPA, similar to alteplase but modified in order to achieve a longer half-life (approximately 13–16 minutes), as well as improved thrombolytic properties by binding to fibrin with a lower affinity than alteplase.

A randomized, feasibility study using primates demonstrated preliminary support that IA (intra-arterial) reteplase with IV abciximab, as well as IA reteplase without IV abciximab, was effective in obtaining recanalization in an intracranial thrombosis model [27]. A prospective, nonrandomized, open-label trial was conducted to evaluate the safety of an escalating dose of reteplase in conjunction with i.v. abciximab in patients with AIS (3–6 h after symptom onset). The authors hypothesized higher rates of recanalization and improved clinical outcomes were due to the combination of medications that lyse fibrin and prevent aggregation of platelets [9]. Patients had NIHSS scores of 4 or greater or 23 or less and arterial occlusion demonstrated by diagnostic angiography. Partial or complete recanalization was observed in 13 of the 20 patients. Thirteen patients demonstrated early neurological improvement, and favorable outcome at 1 month was observed in six patients. In this study, a combination of intra-arterial reteplase and i.v. abciximab was safely administered to patients with ischemic stroke presenting between 3 and 6 hours after symptom onset.

Reteplase was first studied in a primate model of intracranial thrombosis [27], but the study was nonrandomized and did not meet RIGOR [28] and STAIR criteria. RIGOR and STAIR criteria are a set of recommendations for performing better research, including the encouragement of randomization, blinded studies, power analyses, and the like [28].

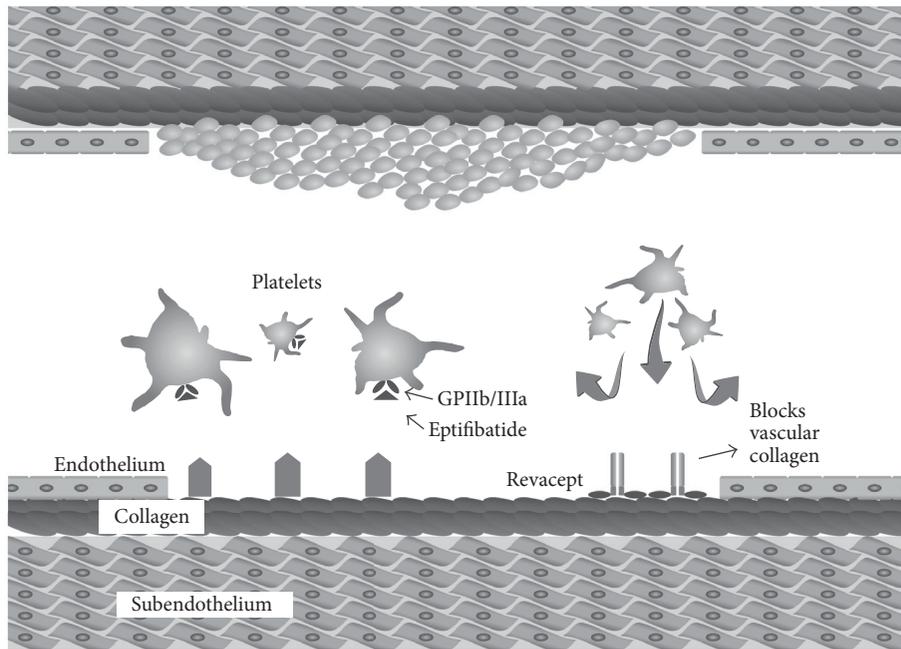


FIGURE 2: The drugs eptifibatid and revacept act by decreasing platelet aggregation and further formation of a clot.

TABLE 1: This table summarizes the hemostatic drugs, details of clinical trials completed in AIS, route (i.v.: intravenous), phases, number of patients enrolled, and clinical trial number.

Summary of antithrombotic drug trials						
Drug	Dose	Time window	Phase	Number of patients	Clinical trial number	Citation
Eptifibatid (CLEAR)	r-tPA (0.3 mg/kg versus 0.45 mg/kg) + eptifibatid (75 µg/kg bolus followed by 0.75 µg/kg/min infusion for 2 hours) or r-tPA (0.9 mg/kg)	3 hours	1/2	10	NCT00250991	[3]
Eptifibatid (CLEAR-ER)	0.6 mg/kg r-tPA + eptifibatid (135 µg/kg bolus and a 2-hour infusion at 0.75 µg/kg per minute) versus r-tPA (0.9 mg/kg)	3 hours	2	126	NCT00894803	[5]
Eptifibatid (CLEAR-FDR)	i.v. bolus of 135 µg/kg eptifibatid followed by an i.v. infusion of 0.75 µg/kg/min eptifibatid for 2 hours	3 hours	2	27	NCT01977456	[6]
Revacept	Single i.v. of 10, 20, 40, 80, or 160 mg	Laboratory, clinical exams	1	30	NCT01042964	[7]

3.2. *Tenecteplase*. Tenecteplase is a genetically engineered mutant tPA which may possess some advantages over alteplase, such as a longer half-life, more resistance to plasminogen activator inhibitor, more fibrin specificity, and producing less systemic depletion of circulating fibrinogen. These advantages lead to faster perfusion and lower incidences of sICH [8]. In a New Zealand study, Parsons et al. set out to determine whether alteplase or tenecteplase had superior outcomes. In this phase IIb trial, 75 AIS patients received treatment in the form of alteplase (0.9 mg/kg) or tenecteplase, low dose (0.1 mg/kg) or high dose (0.25 mg/kg). The baseline NIHSS scores of all patients are approximately

14 (+−2.6). Treatment was administered within 3-4 hours of stroke onset. Outcome measures included the percentage of perfusion of the lesion measured by MRI, clinical and neurological improvements measured by a change in NIHSS score, and changes in mRS scores at 24 hours. There was a significant improvement in the tenecteplase groups, with 79% average reperfusion, compared to 55% in the alteplase group, as well as 64% of tenecteplase group patients having a reduced NIHSS score by 8 or greater, compared to 36% of the alteplase group. The authors conclude that phase III trials are appropriate based on their findings [10]. A Scottish study also tested tenecteplase versus alteplase with the endpoint

TABLE 2: This table summarizes the thrombolytic drugs, details of clinical trials completed in AIS, route (i.v.: intravenous; i.a.: intra-arterial), phases, number of patients enrolled, and clinical trial number.

Drug	Dose	Summary of thrombolytic drug trials				
		Time window	Phase	Number of patients	Clinical trial number	Citation
Retepase + abciximab	0.25 mg/kg bolus of abciximab i.v. + 0.125 mcg/kg/min infusion for 12 hours i.a. reteplase in boluses of 0.25 units (5 minutes) proximal to the thrombus + incremental doses	3–6 hours after symptom onset	1	20	FDA Protocol Number 9180	[9]
Tenecteplase	0.25 mg/kg tenecteplase 0.1 mg/kg tenecteplase 0.9 mg/kg alteplase	6 hours after symptom onset	2b	75	New Zealand Clinical Trials Registry: ACTRN12608000466347	[10]
Tenecteplase	0.25 mg/kg tenecteplase 0.9 mg/kg alteplase	4.5 hours after symptom onset	2	104	NCT01472926	[11]

being the percentage of penumbra salvaged seen via CT at 24–48 hours after treatment. In a phase II, prospective, randomized, open-label, blinded study, 104 patients were treated with the standard dose of alteplase or high dose (0.25 mg/kg) of tenecteplase within 4.5 hours of stroke onset. Of the 104 patients enrolled, 35 tenecteplase and 36 alteplase patients contributed to the endpoint. No difference was found between the groups in salvaged penumbra, incidences of sICH, or other adverse effects [11]. Table 2 summarizes details of completed clinical trials testing these thrombolytic drugs.

4. Endovascular Procedure Trials

The advantages of intra-arterial treatment in AIS with anterior large-vessel occlusion were proven in many randomized controlled trials (RCTs) and consolidated into clinical practice. Acute large-vessel occlusions have less benefit from r-tPA treatment. Critical time points during intra-arterial treatment include time to angiography/perfusion imaging and time to start revascularization with r-tPA in order to get improved neurologic outcomes. There are 5 prospective randomized open blinded endpoint- (PROBE-) designed trials published between 2014 and 2015 in acute large-vessel anterior circulation ischemia and they focus on 90-day outcomes. According to results of these trials, endovascular treatment groups have higher outcome scores (mRS 0–2) as a primary endpoint. These 5 trials are the following.

ESCAPE had 315 patients studied with multiphase CTA to determine the intracranial collateral circulation. The start of CT to groin puncture was less than 60 minutes and time to first reperfusion was less than 90 minutes. Different from other RCTs, the time window in the study was 12 hours; however the study was not powered to evaluate endovascular therapy between 6 and 12 hours after symptom onset because of insufficient patient number [8].

Campbell BC and colleagues published the EXTEND-IA trial. The study was stopped early because of efficacy at 70 patients. Using advanced penumbral imaging techniques,

control patients had lower median NIHSS scores compared to the endovascular group (13 versus 17, resp.). The other 4 RCTs mentioned in this section have used ASPECT scoring for infarct region, which was not used by the EXTEND-IA trial. This trial focused on patients with ischemic core volume of less than 70 mL who received i.v. r-tPA within 4.5 hours' time window. Similar to the ESCAPE trial, internal carotid artery or proximal MCA occluded patients were enrolled [29].

The SWIFT PRIME trial had an 88% rate of complete reperfusion, the highest rate in comparison to the other RCTs mentioned in this section. This finding may be associated with exclusion of patients with extra cranial carotid occlusion. Only EXTEND-IA and SWIFT PRIME trials have 100% of their active control groups receiving i.v. r-tPA and comparing them to intra-arterial recanalization with i.v. r-tPA in the study groups [30].

In October 2015, REVASCAT was published. It had the longest median time period of 269 minutes up to recanalization. Patients with confirmed revascularization after r-tPA were excluded from this study. Patients who have failed treatment for large-vessel occlusion after r-tPA, confirmed on computed tomographic angiography, were the focus of endovascular treatment. This trial has the lowest sICH rates among the other 4 endovascular trials, further supporting the efficacy of endovascular treatment up to 8 hours; however the study was terminated before completing planned enrollment [31].

A 2015 meta-analysis sought to compare outcomes in the form of functional independence (mRS scores between 0 and 2 at 90 days) in 8 clinical trial studies, including SYNTHESIS, MR RESCUE, MR CLEAN, ESCAPE, EXTEND-IA, and REVASCAT. Endovascular therapy treatment patients had better outcomes across the board. There were no significant differences in mortality or incidence of sICH between endovascular therapy groups and medication only groups. Authors concluded that the best course of action is a treatment that combines standard medical treatments with endovascular therapy for best outcomes for qualifying patients [32].

5. Neuroprotection

5.1. Lovastatin. Beside cholesterol reducing effects, statins are considered to have favorable impact on blood brain barrier, oxidative stress, cerebral blood flow, and inflammation according to previous studies. Experimental studies showed several statins have neuroprotective effects on neuronal injury and infarct size in rodent models of AIS when given both before and after AIS [33, 34]. However, a meta-analysis in 2011 by Squizzato et al. demonstrated that in 8 randomized clinical trials involving 625 participants statin treatment did not reduce all-cause mortality compared with placebo or no treatment in the 431 patients enrolled in 7 out of the 8 studies. This was explained as due to inadequate data [31].

The Neuroprotection with Statin Therapy for Acute Recovery Trial (NeuSTART) is a nonrandomized, single group assignment, phase I B dose-escalation study focused on testing the hypothesis that short-term statin therapy at maximally effective doses provides neuroprotection based on animal studies. In this trial AIS patients were treated within 24 hours of symptom onset. The maximum tolerated lovastatin dose was 8 mg/kg/day, which is a higher dose than currently approved by the FDA, and they found 13% toxicity. No clinical liver disease, myopathy, or creatine phosphokinase elevations were reported. This trial showed an appropriate treatment period of 3 days after an AIS for that dose. They found a significant decrease in TNF- α receptor 1 (TNFR1) levels associated with dose increase but no effects on CRP, IL-6, or TNF levels. In addition, no significant dose-related effect on platelet aggregation was detected. The limitations of this trial include insufficient patient number and no neurological outcome reported during dose escalation. On the other hand, it is encouraging to see that a dose higher than the current oral maximum was tolerated with low toxicity. Thus, this dose could be used in future placebo-controlled randomized trials for various outcomes [35].

Researchers are recruiting individuals for a phase II clinical trial with low and high dose lovastatin comparing to placebo within 24 hours of symptom onset. The inclusion criteria for this trial differ from the prior trial in that patients will receive both standard dose i.v. t-PA and/or mechanical interventional procedures in their respective groups. The study will focus primarily on musculoskeletal and hepatic toxicity with a 3-month follow-up period. Secondary targets are neurological outcomes and effects on inflammatory markers [14, 35].

5.2. Donepezil. Donepezil is a reversible, selective acetylcholinesterase inhibitor broadly used in the treatment of Alzheimer's dementia [36]. Enhancement of the cholinergic system showed beneficial effects in trials of chronic stroke [37, 38] and poststroke recovery [39–42] and in experimental stroke models [43]. These results led to the open-label study conducted by the Mayo Acute Stroke Trial for Enhancing Recovery (MASTER) Study Group. Thirty-three patients with AIS were treated with donepezil within 24 hours after event. Donepezil was demonstrated to be safe and tolerated at an initial dose of 5 mg daily for the first 4 weeks; then it was increased to 10 mg per day. Neurologic, cognitive, functional,

and psychological outcomes 90 days after stroke with donepezil treatment compared to the National Institutes of Health Stroke Scale (NINDS) r-tPA trial data showed a tendency for favorable outcomes [44]. The evidence was satisfying enough for this research group to plan further investigation of donepezil in AIS management in a randomized study.

Limitations to the MASTER Study include the fact that it was single-armed and open-label study conducted with 33 patients and only 76% of the enrolled patients completed the 90-day treatment. There was no concurrent control group, and instead the results were compared to data from the National Institute of Neurological Disorders and Stroke (NINDS) r-tPA trial. Patients with probability of AIS were also included. The null hypothesis with the preset level of significance ($\alpha = 0.10$) for continuation in a randomized controlled study was just barely met. Table 3 provides details about clinical trials investigating the neuroprotective drugs.

6. Neurogenesis

6.1. Granulocyte Colony-Stimulating Factor (G-CSF). Granulocyte colony-stimulating factor (G-CSF) is a growth factor cytokine and hormone that stimulates the bone marrow to produce granulocytes and stem cells. Genetically engineered recombinant human G-CSF (such as leucostim and filgrastim) is frequently used in the treatment of neutropenia associated with chemotherapy or bone marrow transplantation. Their physicochemical characteristics and specific biological activity are equal. Preclinical results from numerous studies in stroke models statistically confirmed that recombinant G-CSF has both neuroprotective and neuroreparative effects, activating antiapoptotic, antioxidative, and anti-inflammatory signaling pathways, and stimulating angiogenesis [45]. For further translation into the clinical setting, several safety and feasibility studies were conducted using different dosages and different recombinant G-CSF analogs as additional treatment in AIS patients.

A Russian research group evaluated leucostim effects on blood cell count after AI and specifically focused on leukocytes and progenitor stem cells. In their randomized controlled study leucostim was given s.c. 10 mg/kg/day in addition to conservative treatment. They concluded treatment within 48 hours after AIS for 5 days to be safe, with no significant difference in the outcome (NIHSS, BI, Glasgow Outcome Scale) compared to the control group shown [25]. The study group was small with only 20 patients enrolled, and only six patients completed the full G-CSF treatment. The treatment was given in addition to conventional therapy, but patients with thrombolysis were excluded from this study. Also, there was no placebo given to the control group. For the safety analysis all patients were included, but for the efficacy analysis only the patients who completed the 180-day follow-up were included.

A Chinese randomized controlled trial was conducted on ten patients with middle cerebral artery infarction. Seven were treated with filgrastim 15 μ g/kg/d s.c. for 5 days. Outcome was assessed with the National Institute of Health Stroke Scale (NIHSS), European Stroke Scale (ESS), European Stroke Scale Motor Subscale (EMS), and Barthel Index

TABLE 3: This table summarizes neuroprotective drugs, details of clinical trials completed in AIS, route (i.v.: intravenous; p.o.: per-oral), phases, number of patients enrolled, and clinical trial number.

Summary of neuroprotective drug trials						
Drug	Dose	Time window	Phase	Number of patients	Clinical trial number	Citation
GSK249320	i.v. escalation cohorts 1, 5, and 15 mg/kg	24–72 hours	2	42	NCT00833989	[12]
GSK249320	i.v. escalation doses of 0.04, 0.4, 1.2, 3.5, 10, and 25 mg/kg	Healthy volunteers	1	47	NCT00622609	[13]
Lovastatin	1, 3, 6, 8, and 10 mg/kg per day for 3 days	24 hours	1	33	NCT00243880	[14]
Donepezil	5 mg/day p.o. for 30 days, increased to 10 mg/day for 60 days	≤24 hours	2a	33	NCT00805792	[15]

(BI). Initial treatment within a window of 7 days demonstrated beneficial effects on outcome compared to nontreated patients, while G-CSF administration within 24 hours after stroke was superior. Furthermore, every patient underwent MRI and PET at the 12-month follow-up, showing increased metabolic activity in the peri-infarction area after G-CSF treatment [21]. There was no mention of whether thrombolysis was performed in these patients. This study did not prespecify a baseline infarction volume.

In Germany, 20 patients with AIS were enrolled in an open-label dose-escalation study and treated with three dosages of filgrastim s.c. (2.5, 5, or 10 $\mu\text{g}/\text{kg}/\text{daily}$ for 5 days) within 12 hours of onset [46]. Full standard care was given, including thrombolysis with i.v. r-tPA (0.9 mg/kg) within 3 hours of stroke. Although the inclusion criteria specified ischemia in the middle cerebral artery (MCA) territory, one patient with anterior cerebral infarction and two patients with vertebrobasilar territory infarction were regarded as a minor protocol violation and not excluded. Also, because the age window was broad (age over 18 years), two patients in their thirties were included. In small study groups this can have a big impact. Infarction area was evaluated with voxel-guided morphometry and neurological outcome was assessed (NIHSS, mRS, BI). Neurophysiological testing showed a time-dependent improvement, but there was no comparison to a control group. Four patients with adverse side events were suggested to be unlikely related to G-CSF.

In a Japanese prospective phase I study, filgrastim was given to 18 patients in three doses i.v. (150, 300, or 450 $\mu\text{g}/\text{kg}/\text{day}$) at two time points after event (24 hours or at 7 days). The lower dosages produced no increase in leukocytes and were safe and well tolerated. Past 90 days, neurological outcomes (NIHSS, mRS, BI) were improved in those with G-CSF treatment within 24 hours compared to treatment starting 7 days later [22]. This study is limited due to the small numbers of patients ($n = 3$ for each group and time point). The aim to evaluate two treatment windows and different dosages in a single phase I study with only 18 enrolled patients may have been too optimistic. Additionally, the conventional stroke therapy regimen such as antiplatelet or anticoagulant agents in the acute phase differed from the subacute phase group.

Two German research groups conducted two multicenter trials with large cohorts. The first was the AXIS-2 trial that enrolled 44 patients in a randomized, placebo-controlled dose-escalation study analyzing four i.v. dosages of filgrastim over 3 days (30, 90, 135, and 180 $\mu\text{g}/\text{kg}$ cumulative doses). The treatment window was 12 hours after stroke [23]. Elevation of leukocyte counts never reached the prespecified level for termination and decreased spontaneously at the end of therapy; however, no harmful effects were observed at the 3-month follow-up. These results led to the subsequent multicenter, randomized, and placebo-controlled trial with a cohort of 324 treated patients. Filgrastim was administered within 9 hours after the stroke event in a cumulative dose of 135 $\mu\text{g}/\text{kg}$ i.v. over 72 hours to patients with medium and large ischemic infarctions in MCA territory. A tendency for radiologically reduced infarction volume was observed, but the study failed to prove significant beneficial effects in outcome (NIHSS, mRS, BI) after 3 months [24], despite promising preclinical and clinical data. These results demonstrate the challenges in translating findings from the animal laboratory to clinical stroke patients.

In summary, there is no clinical trial data published to date that shows significant successful effects of G-CSF treatment in a large cohort of stroke patients. Leukocyte counts were temporarily increased during treatment, yet no harmful effects were observed in any trial. Mobilized hematopoietic cells in peripheral blood were elevated after recombinant G-CSF treatment, proving effects of treatment. Early administration of G-CSF treatment of AIS within 12 to 24 hours after onset has only shown a trend towards beneficial effect on outcome.

7. Neuroinflammation

7.1. SA4503. Sigma receptors are involved in several central nervous system (CNS) disorders. More specifically, sigma-1 receptors found in the endoplasmic reticulum are binding sites which may have an effect on neurotransmitter systems via calcium signaling. Cutamesine is a ligand selective for this receptor and may oversee some neuroprotective effects in the framework of neurodegenerative diseases [47]. A growing

body of evidence indicates the involvement of sigma-1 receptors in the mechanisms of various therapeutic drugs; examples include donepezil and neurosteroids [48].

Previous studies prove that IL-1 β , TNF- α , and IFN- γ levels are elevated in the ischemic hemisphere following stroke. Sig-1R activation reduces microglia activity and release of TNF- α , IL-10, and nitric oxide in lipopolysaccharide activated cells. It also helps to stabilize some intracellular proteins in response to cellular stress and induces bcl-2 in reactive oxygen species dependent apoptosis [49]. Ruscher and colleagues demonstrated that treatment with SA4503 in a rat MCAO model had no effect on proinflammatory cytokines in the infarct core or peri-infarct region but resulted in a significant increase in Ibal expression in the infarct core [50].

Urfer and colleagues conducted a multicenter, randomized, double-blind, placebo-controlled phase II study with 60 patients giving once daily low and high dose oral cutamesine treatment 48–72 hours after AIS for 28 days. It was reported that the average time from stroke onset to starting treatment was 60 hours. At the end of the 28th and 56th day there was no significant neurological improvement between low and high dose regimens compared to the placebo group. However, patients with a baseline NIHSS score of ≥ 7 and 9 showed a statistically significant difference between the 3 mg/d cutamesine group and placebo from baseline total NIHSS at the end of treatment [49]. The authors pointed out that patients treated with 3 mg/d cutamesine had a better 10-minute walk test compared to placebo at the 28th and 56th day but with no statistically significant difference, even though these patients received similar hours of daily rehabilitation therapy. In this clinical trial, treatment was started in the subacute phase of stroke. Consequently, the patient population included mostly neurologically stable patients compared to those in the acute phase. Treatment was applied long after thrombolytic therapy; thus patients had higher baseline neurological scores before initiation of oral cutamesine. This likely explains why oral cutamesine treatment has better outcomes in patients with higher baseline neurologic score. The results of this trial might support the use of cutamesine as a supportive drug along with physical therapy in patients who have moderate neurological status after AIS.

In the following sections, we will discuss different groups of neuroprotective agents according to their mechanism of action such as excitotoxicity and oxidative stress reduction.

8. Excitotoxicity

8.1. Caffeinol. Caffeinol includes caffeine plus ethanol and acts through central adenosine, gamma-aminobutyric acid (GABA) A, and N-methyl-D-aspartate (NMDA) receptors [51]. Belayev and colleagues applied caffeinol in ischemic rats starting at 15 minutes after reperfusion with a 2.5-hour infusion. They reported a significant decrease in cortical infarct volumes and an increase in neurological scores; however, no significant difference was found on subcortical infarct volumes and brain edema [52]. Zhao and colleagues applied caffeinol to rats up to 2-3 h after the onset of transient focal ischemia and found a dramatic decrease in cortical infarct

volume [53]. This in vivo excitotoxicity model based on intracortical infusion of NMDA and a model of reversible focal ischemia demonstrated NMDA receptor inhibition as one of the possible mechanisms of caffeinol anti-ischemic activity. They also emphasized the antiexcitotoxic effect of caffeinol was not as potent as its anti-ischemic effect. Conversely, in a rabbit small clot embolic stroke model, caffeinol treatment was administered as an infusion or as multiple bolus injections but no improvement in behavioral rating scores following an embolic stroke was detected [54]. Lapchak and colleagues explained that the conflict regarding rat studies is due to the fact that ischemic lesion in this rat model not only is restricted to the cerebral cortex but also includes subcortical regions. This observation is compatible with the study by Belayev and colleagues, which found no caffeinol neuroprotective effects on subcortical infarct volumes. In the same study, Lapchak and colleagues combined caffeinol administration with low-dose t-PA. However, this combination reduced neurologic scores and raised the incidence of intracerebral hemorrhage, although not significantly.

Martin-Schild and colleagues designed a phase I, non-randomized, single group assignment trial with 20 patients and aimed to investigate if caffeinol (caffeine 8-9 mg/kg + ethanol 0.4 g/kg IV X 2 h, started 4 hours after the onset of symptoms) and hypothermia (starting 5 hours after symptom onset, continued for 24 hours reaching a target temperature between 33 and 35°C, and followed by 12 hours of rewarming) could be administered safely together with t-PA treatment in first 3-4 h of acute ischemic stroke. Neurologic improvement in NIHSS scores and adverse events related to t-PA was not higher than the expected rate. Main weaknesses of the trial include inadequate number of patients, no placebo group, and only about 1/2–2/3 of the patients being reported to have reached the targeted post-caffeinol caffeine and ethanol levels used in previous rodent studies [17].

9. Oxidative Stress and Cytoprotection

9.1. Edaravone. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a radical scavenger that inhibits nonenzymatic lipid peroxidation and lipoxygenase pathways. It has neuroprotective roles against ischemia or reperfusion-induced vascular endothelial cell injury as well as against delayed neuronal death, brain edema, and neurological deficits. Edaravone was shown to avoid extravasation of r-tPA administered in an ischemic stroke rat model thereby reducing the incidence of hemorrhagic transformation. In an observational study, edaravone treatment was found to have a low frequency of hemorrhagic transformation and mortality. In a propensity-matched analysis study, Wada and colleagues reported that combining r-tPA treatment with edaravone improved mRS scores at discharge after acute ischemic stroke. No significant effect was found on 7-day mortality, hemorrhagic transformation, or length of hospital stay [55].

An ongoing phase II clinical trial (NCT02430350) is focusing on different doses of edaravone injection for 14 days following AIS. The investigators are evaluating neurological outcomes, cognitive assessment, and safety of the drug out to 90 days. In Japan a regimen combining r-tPA + MCI-186 has

been used with 30 mg i.v. MCI-186 over 30 minutes twice per day for 14 days following AIS.

Kaste and colleagues conducted a phase II multicenter, double-blinded, placebo-controlled clinical trial in 36 ischemic stroke patients. Two different doses at 24 h after stroke were planned. Various acute adverse effects were reported in 88.9%. Hypertension was found to be the most frequent hemodynamic adverse effect in both groups. Atrial fibrillation was more frequent in high dose treatment group but there was no significant difference for other adverse effects between the 2 dose regimens. Both dosing regimens raised the plasma concentration to a plateau within 24 hours; however, this trial was lacking in detailed pharmacokinetic parameters. The authors analyzed neurological impairments in the full analysis population of which all patients had a baseline NIHSS score of 3–15. There were no significant differences in neurological improvement results between the placebo group and the 2 dosing regimens at 72 h, 120 h, 31 and 87 days [18]. The main limitation of this trial is inadequate patient enrollment compared to 252 patients that enrolled in the Japanese r-tPA + MCI-186 study. Furthermore, since Kaste and colleagues reported no significant acute neurologic improvement at the end of the treatment (72 h) after stroke, two possible perspectives can be considered. First, acute MCI-186 infusion might have better clinical effects in the chronic period (more than 3 months) after stroke and it deserves investigation. Second, researching the effects of long term MCI-186 treatment following acute ischemic stroke (14-day treatment) as done in the Japanese drug approval protocol seems warranted. Furthermore, due to high acute adverse effects, oral treatment regimens might be more feasible than i.v. infusion in clinical trials.

9.2. Glyburide (RP-1127). Glyburide (RP-1127), also known as Glibenclamide (Gbc), is an antidiabetic drug in the class of sulfonylureas and functions by blocking either adenosine triphosphate- (ATP-) sensitive K^+ channels and/or sulfonylurea receptor 1 (SUR1) in various experimental ischemic models [56]. KATP channels are rapidly activated in response to an increase of intracellular ADP/hypoxia causing K^+ efflux that leads to inflammation and oxidative stress. Blockade of sulfonylurea receptors with low doses of Gbc reduced cerebral edema and infarct volume and decreased mortality by 50% in ischemic stroke rat models. Sulfonylurea receptors are associated with the astroglial NCCa-ATP channel [57] and microglial KATP channel. Abdallah and colleagues exhibited Gbc effects on diminishing neutrophil recruitment, recovering prooxidant/antioxidant balance, decreasing inflammatory mediators, increasing IL-10, and mitigating reperfusion-induced hypoglycemia in a study using Gbc pretreatment and Gbc 10 minutes before ischemia-reperfusion [58].

Sheth and colleagues conducted an open-label, single group assignment, phase II trial (GAMES-Pilot) in 10 severe anterior circulation ischemic stroke patients, 90% of whom had high NIHSS scores and received r-tPA. They aimed to investigate feasibility and tolerability of RP-1127 in this setting. The study results included reduced hemispheric volume, hemorrhagic transformation, lesion growth, and mortality, along with improved neurological outcomes. Limitations to

this trial include small study size and no placebo control group [59]. The same group conducted a retrospective, case-control clinical trial using patients from the GAMES-Pilot trial, using matched control cohorts who were in two different stroke protocols at a single institute. They aimed to evaluate the effect of i.v. Gbc on vasogenic and cytotoxic brain edema with MMP-9. No difference between apparent diffusion coefficient (maps sensitive to cytotoxic edema in early stages of infarction) values at day one was found, but lower MMP-9 levels were detected in i.v. Gbc patients [59]. As the study highlighted, the main disadvantages in this trial are the insufficient number of patients with different baseline data between control groups and timing heterogeneity for MRI and blood sampling between groups. In addition, the GAMES-Pilot study included only large hemispheric infarction and it is challenging to adapt these results to ischemic stroke patients. On the other hand, it is important to emphasize that i.v. Gbc treatment was associated with lower hemorrhagic transformation and MMP-9 levels.

A recent and more promising GAMES-RP trial with the same research group was designed as a randomized, prospective, placebo-controlled, double-blind phase II trial of RP-1127 in subjects with a severe anterior circulation ischemic stroke. Important criteria for inclusion are patients with r-tPA treatment and 10-hour treatment window for i.v. Glyburide injection. The author's rationale was due to the GAMES-Pilot study data that revealed that RP-1127 had positive effects up to 10 h. The pivotal objective of GAMES-RP study is to specifically purpose patients who are prone to malignant edema after ischemic stroke.

9.3. 3K3A-APC. Activated protein C (APC) is derived from plasma protease zymogen, provides neuroprotection, and has antithrombotic and anti-inflammatory effects. APC stimulates multiple cytoprotective pathways via the protease activated receptor-1 (PAR-1) reducing ischemia induced injury [60]. 3K3A-APC is a recombinant variant of human APC that was designed to preserve activity at PAR-1, such as cell signaling actions, but with reduced anticoagulation. Although reduced anticoagulation may seem counterintuitive, when APC was used for the treatment of sepsis, serious bleeding was a common side effect. Thus, a modified APC (3K3A-APC) was created to address this issue. A phase I trial [26, 61] characterized pharmacokinetics and anticoagulation effects demonstrating that this protein was well tolerated at multiple doses as high as 540 g/kg 2x daily for 3 days. In addition, it also confirmed the drug had minimal coagulopathy effects. The study established tolerability and safety of the 540 mg/kg dose as only nonserious side effects were observed. 3K3A-APC shows synergistic efficacy in combination with r-tPA, with hemorrhage rates reduced after r-tPA treatment in combination [62–64]. However, when used in combination, 3K3A-APC shows no effect on r-tPA lytic effect [65].

3K3A-APC was shown to protect aging female mice as well as comorbid spontaneously hypertensive rats from ischemic stroke, as well as to extend the therapeutic window of r-tPA [61]. While all of this data is reassuring, there still remains uncertainty regarding whether the drug will work in humans, as there is no valid guide to translate effective

serum concentrations in rodents to dosing in humans, as highlighted by the authors. In the past, many neuroprotectant agents have failed partly because serum concentrations were significantly lower in subjects than in the successful animal studies. It is reassuring that the maximally tolerated dose in healthy volunteers is well above the dose (200 g/kg) shown to be maximally protective in animals [66, 67].

There is currently an ongoing phase II trial, RHAPSODY, to evaluate the safety and efficacy of 3K3A-APC in multiple intravenous doses. This double-blinded, randomized study aims to measure adverse events that meet dose limiting toxicity, as well as the maximum observed plasma concentration of the target drug. Participants suffering from ischemic stroke will be treated with 3K3A-APC or a placebo, as well as either r-tPA or mechanical thrombectomy, or both treatments. The RHAPSODY trial is scheduled to be completed in March 2017 (NCT02222714).

Further clinical development should include a tolerability study in stroke subjects [26, 61]. In summary, the molecule demonstrates it was engineered for minimal coagulopathy.

Table 4 provides details about clinical trials investigating the drugs.

10. Conclusion

In this review, a number of clinical trials resulting in positive findings were identified. These hold the potential for future phase 3 studies in the area of AIS. The combination of eptifibatide with r-tPA has brought great hope to the future treatment of AIS. The "CLEAR" trials proved safety and tolerability up to 36 hours with dose escalation. Studies on reteplase with abciximab provided optimistic results regarding the possibility of increasing reperfusion after AIS. Furthermore, reteplase has joined the list of drugs being considered for use in the clinical setting. There is potential for synergy of drugs that inhibit platelet adhesion to the subendothelium and thrombolytic agents thereby improving clot degradation.

A very common issue is the challenge of enrolling a sufficient number of representative patients with comparable characteristics. Therefore, the results represent more a preliminary estimation than a representative evaluation. Some trials lack a control group. As in many stroke trials NIHSS, mRS, and BI evaluation does not necessarily correspond with infarction volume and overall tissue damage. Furthermore, NIHSS scores at presentation should be similar in each arm, as it is a major contributor and predictor of clinical outcomes.

When considering the results of the phase I/II clinical trials discussed in this review, many challenging unanswered questions still remain. Specific drugs offer higher potential for clinical translation, including revacept and eptifibatide. The pursuit of a better therapy and continuation of preclinical and clinical trials is imperative in order to reach a new level of treatment options for acute ischemic stroke. Future phase 3 clinical trials will be observed closely to determine which of these therapies hold the key to advances in treating AIS.

It is important to highlight that neuroprotective treatment strategies after acute ischemic stroke should focus on the progression of destructive molecular and biochemical cascades

following cerebral hypoperfusion and anaerobic glycolysis. The aims of these neuroprotective approaches are to prevent ischemic brain injury from progressing into infarction. The ischemic penumbra is a conceivably recoverable region around the ischemic core where collateral cerebral blood flow supports neuronal perfusion. Thrombolytic and endovascular treatment provides reperfusion and attenuates the development of irreversible neuronal injury in penumbral region. Ischemic cascades have various spots in which its inhibition might increase the effectiveness of perfusion with thrombolytic and endovascular strategies. Time window is one of the crucial factors determining the treatment efficacy, particularly in thrombolytic and antithrombotic drugs. When eptifibatide was applied within 3 hours and reteplase + abciximab within 3 to 6 hours, neurological improvement was reported. Similarly time windows in recent endovascular clinical trials treatments are from 6 to 12 hours.

Possible pathophysiological steps that can be manipulated following anaerobic glycolysis are ionic imbalance, oxidative effects of free radicals, excitotoxicity, neuroinflammation, and apoptosis. The neuroprotective drugs are generally applied 24 to 72 hours after ischemic stroke onset and have favorable outcome in trials. This is due to the fact that their mechanism does not depend on restoring cerebral perfusion and therefore differs from antithrombotic and fibrinolytic drugs. Thus, encouraging treatment strategies should focus on combination therapies with fibrinolytic drugs in early stroke and neuroprotective drugs in 24 to 72 hours.

There is growing experimental evidence regarding the association between ischemic brain injury, neuroinflammation, and endogenous neurogenesis during the recovery period. Ischemic injury stimulates neurogenesis in the subventricular zone and subgranular layer of dentate gyrus. For that reason, the recombinant form of G-CSF, by stimulating neurogenesis, is suggested to be a future additional treatment to standard of care.

Given the plethora of information from phase I and phase II clinical trials in acute ischemic stroke, it is imperative that researchers do not dismay over negative results of these clinical trials but keep looking forward to more designs and new medications used in other areas of medicine that may benefit the brain via their mechanism of action. This will not only improve health and survival but also improve neurological outcome following AIS. In addition, future clinical trials should include a larger number of patients, different time windows, fulfillment of the planned enrollment, multicenter design, application of r-tPA, use of advanced neuroimaging techniques to target penumbra, and a clear separation of patients before treatment according to neurological scores for accurate correlation between treatment and prior neurologic severity. The STAIR and RIGOR criteria should be followed.

It is well known in the literature that drug development strategies for acute ischemic stroke have failed due to incompatible preclinical models and issues with designing of clinical trials [68]. The main difficulties of drug improvement in acute ischemic stroke trials are the restricted therapeutic window before reperfusion of salvageable brain tissue, clinical misclassification, and inadequate sample size [69]. The evolving of neuroprotective agents is generally sustained by in

TABLE 4: This table summarizes the different drugs, details of clinical trials completed in AIS, route (i.v.: intravenous; s.c.: subcutaneous), phases, n/a: not available, number of patients enrolled, and clinical trial number.

Summary of trials for neuroinflammation, excitotoxicity, and oxidative stress drugs						
Drug	Dose	Time window	Phase	Number of patients	Clinical trial number	Citation
SA4503	Oral treatment of 1 mg/d and 3 mg/d for a period of 28 days	48 to 72 hours	2	60	NCT00639249	[16]
Caffeinol + hypothermia	Infusion of caffeinol (9 mg/kg caffeine + 0.4 g/kg ethanol) over 2 hours	4 hours	1/2	30	NCT00299416	[17]
Edaravone	12.5 mg/37.5 mg/62.5 mg one dose every 12 hours, for period of 14 days	24 hours	2	400	NCT01929096	[18]
RP-1127	3 mg/day i.v. 3 boluses followed by infusion for 72 hours	≤10 hours	2	10	NCT01268683	[19]
RP-1127	i.v. bolus followed by continuous infusion for 72 hours	4.5 hours	2	34	NCT01132703	[20]
Filgrastim	s.c. 15 µg/kg per day for 5 days	7 days	n/a	10	n/a	[21]
Filgrastim	3 i.v. doses (150, 300, or 450 µg/body/day, divided into 2 doses for 5 days)	24 hours and 7 days	1	18	n/a	[22]
AX200 (G-CSF)	4 i.v. doses, total cumulative doses of 30–180 µg/kg over the course of 3 days	<12 hours	2a	44	NCT00132470	[23]
AX200 (filgrastim)	135 µg/kg i.v. over 72 hours	9 hours	2	328	NCT00927836	[24]
Leucostim	10 mg/kg s.c. per day for 5 days	≤48 hours	2	20	NCT00901381	[25]
3K3A-APC	3K3A-APC at 6, 30, 90, 180, 360, 540, or 720 g/kg and 5 doses: 90, 180, 360, or 540 g/kg every 12 hours after safety of the first. g/kg every 12 hours after safety of the first	Measurements at 12 and 24 hours	1	64	NCT01660230	[26]
3K3A-APC	3K3A-APC at 120 ug/kg, 240 ug/kg, 360 ug/kg, or 540 ug/kg	Measurements at 12 hours for up to 5 doses	2	100	NCT02222714	Not yet published

vivo or in vitro data. Reasons of these agents success in animal studies but failure in clinical trials are due to planning and analysis problems in experimental data as well as not effective and broad usage of new neuroimaging modalities in animal studies [70]. Thus, infarct volume declines or neurologic improvements in animal models cannot be reflected into clinical trials outcomes. In order to increase the effectiveness of experimental designs researchers need to integrate the reperfusion treatments and neuroprotective agents together in animal studies with adequate sample size and more specific tissue targeting [71]. Promising future strategies might involve focusing on neurovascular unit injury, response of pericytes, and recruitment of peripheral immune cells following ischemic stroke [72].

Before starting a clinical trial, it is imperative to know the estimate number of patients and the eligible ones at each site. Main limitations for a clinical trial are age, stroke severity, time to start treatment as well as placebo control, double blind manner, and randomization mechanisms. In addition, it is time consuming, challenging, and expensive task to design, implement, and conduct clinical trials for acute ischemic stroke. For these reasons, organization of clinical trial and safety concerns is addressed to ensure the design and performance of optimal trials to safely evaluate the drug being tested. During the process of drug development, ensure that regulatory guidelines are met and the results of these studies should be revealed to the public. Thus, following Stroke Therapy Academic Industry Roundtable (STAIR) guidelines

would guarantee optimal development of new acute stroke therapies follow specific recommendations that would support the translation from bench side to clinical situations [73]. All these measures would decrease pitfalls and ensure randomization and appropriate sample size and transparent reporting [74].

Over the next five years, research in therapies for acute ischemic stroke will grow along with our understanding of the various pathways in which neural injury as well as neural protection occurs. We predict that r-tPA will not be the only treatment option available for patients presenting with acute ischemic stroke.

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Competing Interests

The authors state no conflict of interests.

Authors' Contributions

Cesar Reis and Onat Akyol contributed equally to this work.

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Review Article

Characteristics of Hemorrhagic Stroke following Spine and Joint Surgeries

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Hemorrhagic stroke can occur after spine and joint surgeries such as laminectomy, lumbar spinal fusion, tumor resection, and total joint arthroplasty. Although this kind of stroke rarely happens, it may cause severe consequences and high mortality rates. Typical clinical symptoms of hemorrhagic stroke after spine and joint surgeries include headache, vomiting, consciousness disturbance, and mental disorders. It can happen several hours after surgeries. Most bleeding sites are located in cerebellar hemisphere and temporal lobe. A cerebrospinal fluid (CSF) leakage caused by surgeries may be the key to intracranial hemorrhages happening. Early diagnosis and treatments are very important for patients to prevent the further progression of intracranial hemorrhages. Several patients need a hematoma evacuation and their prognosis is not optimistic.

1. Introduction

The definition of stroke is “rapidly developing clinical signs of focal disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin” by WHO (The World Health Organization) [1]. Stroke can happen to any person at any time. It happens when poor blood flow to an area of brain, and brain cells begin to die because of lack of oxygen. Abilities such as memory and muscle control controlled by that area of the brain are damaged. According to the National Stroke Association, nearly 800,000 people experience a new or recurrent stroke each year. Stroke is the fifth leading cause of death and the leading cause of adult disability in USA. According to the pathology, stroke can be divided into two main types: ischemic and hemorrhagic. Hemorrhagic strokes are relatively uncommon compared with ischemic strokes. Actually hemorrhagic strokes account for only 15% of all strokes; however 40% of all stroke deaths are attributable to hemorrhagic strokes [2]. The common causes of hemorrhagic strokes include arteriolar hypertensive diseases, burst aneurysm, arteriovenous malformation (AVM), bleeding disorders, head injury, and blood thinners. In addition to this, some clinical scientists find that hemorrhagic strokes sometimes occur after spine and joint surgeries. Although

postoperative stroke rarely happens, it may cause severe consequences and high mortality rates. Chaddock first reported a case of hemorrhagic stroke after cervical laminectomy [3]. Since then, there are several similar cases reported. And most patients in these cases have headache, neurological disorders, and altered level of consciousness after spine surgery. The bleeding site is usually located in cerebellum after spine surgeries. Finally some patients in these cases become disabled and even dead. Hemorrhagic strokes after joint surgeries are also the cause of disability and death. Nevertheless, we know very little about these kinds of hemorrhagic stroke now, and it is necessary to study it in depth. This review summarizes the clinical status, risk factors, pathologic mechanisms, and treatment options of perioperative hemorrhagic stroke after spine and joint surgeries.

2. Methods

A computer-based retrieval was performed to search articles which describe hemorrhagic stroke after spine and joint surgeries published between September 1, 1980, and September 1, 2016, in PubMed database with the key words of “stroke, spine, joint” in English. And 141 articles were found in this way. The inclusion criteria includes the following. (1) Studies should cover the clinical status, risk factors, and treatment

options of hemorrhagic stroke after spine and joint surgeries. (2) Study assumptions and research methods are similar. (3) The patient's diagnosis is clear. The exclusion criteria includes repeated reports, incomplete data, and study defect. After filtering these articles by the inclusion and exclusion criteria, finally, 25 articles are included into this review.

3. Results

3.1. Clinical Status. Postoperative intracranial hemorrhages may happen after spinal surgery in different places in the brain, such as the epidural or subdural space and the supratentorial or cerebellar parenchyma. Although it is a rare complication, it can be related to permanent serious disability [4]. Chaddock reported the first case of remote cerebellar hemorrhage (RCH) of a patient who had undergone a cervical laminectomy in the sitting position. The clinical manifestations of this patient included headache, cerebellar neurological disorders, and altered level of consciousness [3]. After that Mikawa reported the second case: a patient became comatose almost 16 hours after cervical durotomy and revision C1-C2 fusion, as the cerebellar hemorrhage occurred within the first 10 hours after the surgery. From the first case reported to the present, there are 44 published cases of remote hemorrhage reported after spinal surgery. Among these, there are 11 cases undergoing cervical laminectomy, 19 cases undergoing lumbar laminectomy, 10 cases undergoing lumbar spinal fusion, 3 cases undergoing tumor resection, and 1 case undergoing Harrington rod placement [5–10]. In these cases, most patients had clinical manifestations of headache, vomiting, consciousness disturbance, and mental disorders, and the CT scan of these patients showed subarachnoid hemorrhage or hemorrhage of the brain parenchyma. Most patients can recover after active treatment and rehabilitation exercise among these cases; however there are also some patients becoming disabled and even dead (Table 1). In order to verify whether spine surgery is associated with stroke, Jau-Ching Wu conducted a cohort study in Taiwan. In this study, a Taiwan-wide cohort of 1 million people from 2000 to 2005 was divided into the lumbar spinal fusion group and they were followed up for 3 years for stroke. The result shows that patients undergoing lumbar spinal fusion do not have a higher incidence rate of stroke. And the author admits that the result of this study is not convincing enough because of the database limits. So it is still unclear whether spine surgery and stroke are related. Hemorrhagic stroke is also a disastrous complication after joint surgeries such as total joint arthroplasty (TJA). Rasouli et al. made a database review which covered a total of 1,762,496 patients after TJA from 2002 to 2011. After doing a population-based trend analysis, they found that the incidence rate of all perioperative stroke after TJA was nearly 0.14%. And among these perioperative strokes, 20.55% of cases were hemorrhagic stroke. The in-hospital mortality rate was much higher for TJA patients with stroke than patients without stroke (9% versus 0.15%) [11]. Although the epidemiological studies showed a low frequency of perioperative stroke, it is still the leading cause of disability and death for patients after joint surgeries [12].

3.2. Risk Factors. The risk factors of a common stroke include dyslipidemia, hypertension, diabetes mellitus, smoking, and obesity. These risk factors also exist in patients after spine and joint surgeries [13]. For patients after spine surgeries, hypertension and coagulopathy are considered as main risks of hemorrhagic stroke [14]. And low intracranial pressure (ICP) may contribute to intracranial hemorrhages especially subdural hemorrhages [15]. Other important risks include ages of patients and experience of surgeons, and these risks are doubled if a patient once had an experience of a disc surgery [16]. For patients after joint surgeries, the main risks include diabetes mellitus, cardiac diseases, renal diseases, and pulmonary circulation disorders. A history of stroke is not risk factor for contributing to stroke after joint surgeries [11]. There is also a higher incidence of first-ever stroke for patients who are over 65 years old after hip replacement surgeries, and the incidence rate of ischemic stroke is nearly five times than that of hemorrhagic stroke [17]. It is worth mentioning that adiposity-associated risks of women are much greater for ischemic stroke than for hemorrhagic stroke [18].

3.3. Pathologic Mechanisms. The exact pathophysiology of intracranial hemorrhages after spine surgeries is still controversial. However almost all of the theories are associated with cerebrospinal fluid (CSF) leakage which leads to intracranial hypotension [19]. And dura mater tears which can cause CSF leakage are the most common complications in a spine surgery [20]. One of these theories for hemorrhagic stroke after CSF leakage suggests that a downward cerebellar displacement may happen following with the CSF leakage and intracranial hypotension. And the downward cerebellar displacement can lead to the stretching and tearing of cerebral venous system which finally causes hemorrhagic stroke [21]. Another theory believes that the pressure in brain vessels increases after CSF leakage and ruptures the vessels [22]. There are few studies on the pathophysiology of intracranial hemorrhages after joint surgeries because of its low occurrence. So the pathophysiology of intracranial hemorrhages after joint surgeries is not very clear now. With more research conducted and more cases reported, the pathophysiology will be revealed finally.

3.4. Prevention Strategies. According to the currently known risk factors, some prevention strategies can be implemented to prevent patients from hemorrhagic strokes after spine and joint surgeries. Firstly, blood pressure control must be performed throughout the perioperative period especially for those patients with hypertension, because hypertension not only increases the risk of surgery but also increases the incidence of postoperative complications. Secondly, the operator should try to avoid tearing the dura mater during the spine surgery. Once a CSF leakage happens, intracranial pressure monitor will be useful to evaluate the risk of hemorrhagic stroke. And some measures such as dural repair should be done before hemorrhagic stroke happens. For patients after tumor resection, wound suction drainage is a double-edged sword. On the one hand it can reduce intracranial edema, but on the other hand it causes CSF leakage and increases

TABLE 1: Clinical status of hemorrhagic stroke after different types of spine surgeries.

Surgery types	Clinical manifestations	CT appearance	Brain parenchyma hemorrhage location	Treatments	Results	Total case
Cervical laminectomy	10 cases have a serious headache, 1 case has aphasia, and 2 cases have limb motor dysfunction	3 cases show subarachnoid hemorrhage, and 8 cases show brain parenchyma hemorrhage	4 cases locate unilateral cerebellar hemisphere, 3 cases locate unilateral temporal lobe, and 1 case locates bilateral cerebellar hemisphere	8 cases under conservative treatments, 2 cases under dural tear repairing, and 1 case under decompressive craniectomy	9 cases completely recover with no neurologic defect, 1 case recovers with lower limbs spasticity, and 1 case died	11
Lumbar laminectomy	17 cases have headache and nausea, 6 cases have consciousness disturbance, 3 cases have limb motor dysfunction, and 1 has gait ataxia	4 cases show subarachnoid hemorrhage, 12 cases show brain parenchyma hemorrhage, and 1 case shows both of them	8 cases locate unilateral cerebellar hemisphere, 2 cases locate cerebellar vermis, 1 case locates right temporal lobe, and 1 case locates parietooccipital lobes	11 cases under conservative treatments, 4 cases under dural tear repairing, 2 cases under hemorrhage evacuation, and 2 cases under decompressive craniectomy	15 cases completely recover with no neurologic defect, 2 cases died, 1 case has left foot drop and diplopia, and 1 case has cognitive deficit	19
Lumbar spinal fusion	All cases have headache and nausea, 2 cases have dysarthria, 1 case has a speech deficit, and 1 case has consciousness disturbance	1 case shows subarachnoid hemorrhage, and 7 cases show brain parenchyma hemorrhage, and 2 cases show both of them	6 cases locate unilateral cerebellar hemisphere, 1 case locates bilateral cerebellar hemisphere, and 2 cases locate unilateral occipital lobe	7 cases under conservative treatments, 2 cases under dural tear repairing, and 1 case under hematoma evacuation	8 cases completely recover with no neurologic defect, 1 case has speech deficit, and 1 case died	10
Tumor resection	All cases have headache, and 1 has dizziness and vomiting	2 cases show cerebellar hemorrhage, and 1 case shows cerebral hemisphere hemorrhage	2 cases locate unilateral cerebellar hemisphere, and 1 case locates left temporooccipital cortex	All cases under conservative treatments	2 cases completely recover, and 1 case has a slight ataxia	3
Harington rod placement	Headache and vomiting	Subarachnoid hemorrhage and cerebellum hemorrhage	Right cerebellar hemispheres, right ventricle, and subarachnoid spaces	Suboccipital craniotomy	Completely recovered	1

the risk of intracranial hypotension. So the correct choice must be made based on the patient's comprehensive circumstance. Thirdly, early brain CT examinations are necessary for patients with vascular disease or coagulopathy. Finally, the treatment of underlying diseases may also be beneficial to prevent hemorrhagic stroke.

3.5. Treatments. For patients with hemorrhagic stroke after spine surgeries, it is the most important that the CSF leakage and intracranial hypotension must be controlled. So closed wound suction drainage is recommended for spinal surgery especially tumor resection, and the time to stop drainage is determined by the complaints of patients including headache and emesis [8]. In other words, early diagnosis is also very important for the treatment of intracranial hemorrhage after spinal surgery. Brain CT examinations and typical clinical symptoms may contribute to early diagnosis. If the loss of CSF is caused by a dural tear, dural repair and preventing CSF leakage will be useful to prevent intracranial hemorrhages after spine surgeries. Other treatments including symptomatic treatment and supportive care in a common hemorrhagic stroke such as bed rest, clinical intensive observation, fluid therapy, and radiological close monitoring are also necessary for patients with hemorrhagic stroke after spine and joint surgeries [23]. Rational application of mannitol can reduce intracranial hypertension after hemorrhagic stroke happens. Antihypertensive drugs is beneficial to patients with hypertension, whether hemorrhagic stroke happens or not. Other drugs such as hypoglycemic agent for underlying diseases are also necessary. And new drugs such as Fingolimod (FTY720) may be useful to treat hemorrhagic stroke after spine and joint surgeries [24], because the latest laboratory findings and clinical trials strongly support its effectiveness on any kind of hemorrhagic stroke [25].

4. Conclusions

Hemorrhage stroke after spine and joint surgeries is relatively rare, but it may cause serious consequences such as morbidity and mortality. In addition to dyslipidemia, hypertension, diabetes mellitus, smoking, and obesity, the risks of patients after surgeries include coagulopathy and low intracranial pressure. Most patients with hemorrhage stroke after surgeries have clinical manifestations of headache, vomiting, consciousness disturbance, and mental disorders. The bleeding sites are mostly located in cerebellar hemisphere and temporal lobe. Most cases happen several hours after surgeries. And brain CT examinations and typical clinical symptoms may contribute to early diagnosis. A CSF leakage may be the key to intracranial hemorrhages happening. So repairing dural tear or closing wound suction drainage after spine surgeries can be helpful to prevent hemorrhage stroke. Blood pressure control is very important for patients with hypertension. And other treatments such as bed rest, clinical intensive observation, fluid therapy, and radiological close monitoring are also necessary. Most patients can completely recover with no neurologic defect after the conservative treatment. However several patients need a hematoma evacuation. For these patients, their prognosis is not optimistic.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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