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Lead Guest Editor: Yi Yang
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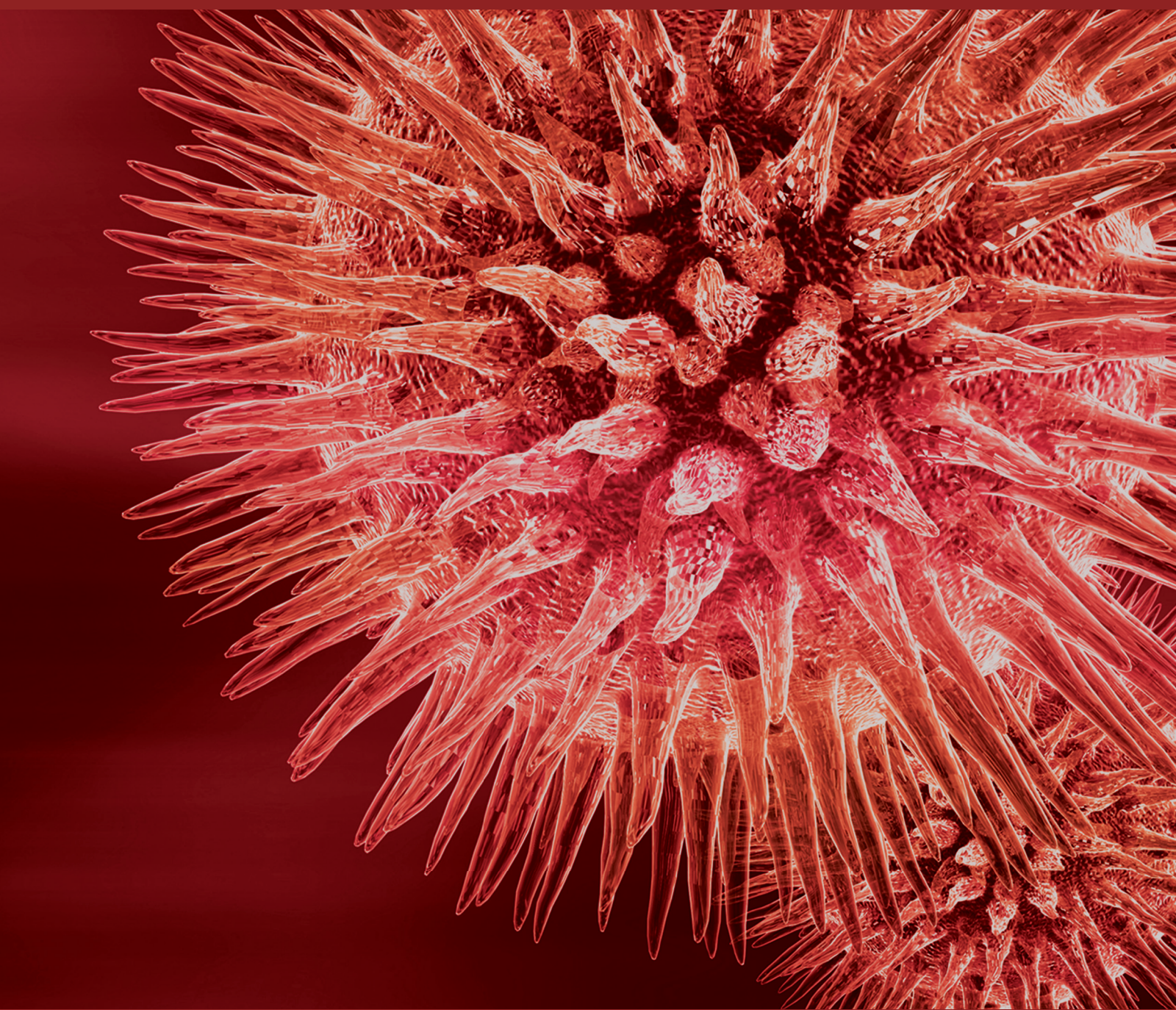

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Contents

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Editorial

Cerebrovascular Regulation in Neurological Disorders

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Cerebrovascular regulation, referring to cerebral autoregulation, cerebrovascular reactivity, and neurovascular coupling, is intrinsic control mechanism of cerebral blood flow and is considered critical for the maintenance of adequate cerebral perfusion. Much previous work has related cerebrovascular regulation to the occurrence and prognosis of a range of neurological disorders, including cerebrovascular diseases, trauma, stroke, cognitive impairment, and neuropsychiatric disorders. Though many studies have identified these links, clinical exploitation of cerebrovascular regulation remains poorly developed. In addition, optimal methods for evaluation and data analysis in clinical studies need to be further discussed. In this special issue, we have brought together several papers that investigate cerebrovascular regulation in neurological disorders. We hope that this issue can encourage further studies on a number of important aspects of cerebrovascular regulation.

The study by J. M. D. van den Brule et al. entitled "Influence of Induced Blood Pressure Variability on the Assessment of Cerebral Autoregulation in Patients after Cardiac Arrest" addresses the influence of increased blood pressure variability on cerebral autoregulation assessment. Cerebral autoregulation measurements are performed in comatose patients after cardiac arrest both at rest and during intervention (tilting of bed). The review of J. M. D. van den Brule et al. entitled "Cerebral Perfusion and Cerebral Autoregulation after Cardiac Arrest" focuses on the alteration of cerebral perfusion and cerebral autoregulation after cardiac arrest, both of which are important in the development of secondary brain damage. The temporal course of cerebral blood flow after the return of spontaneous circulation, as well as cerebral autoregulation after cardiac arrest, is described in this review article.

X. Nie et al.'s review entitled "Futile Recanalization after Endovascular Therapy in Acute Ischemic Stroke" summarizes the predictors of futile recanalization and provides support for clinicians to make informed decisions about vascular recanalization therapy. Futile recanalization is one of the main causes of endovascular treatment failure and poor outcome. Impairment of cerebral blood flow regulation, bad collateral circulation, subacute reocclusion, large hypoperfusion volumes, and microvascular compromise were shown to be involved in this complicated process. The paper of Y. Ma et al. entitled "Pinocembrin Protects Blood-Brain Barrier Function and Expands the Therapeutic Time Window for Tissue-Type Plasminogen Activator Treatment in a Rat Thromboembolic Stroke Model" presents a study of the protective effects of pinocembrin on t-PA administration-induced blood-brain barrier damage in a rat thromboembolic stroke model. The potential mechanisms with which blood-brain barrier damage contributes to hemorrhagic transform after t-PA treatment are still unclear. The disruption of cerebrovascular regulation may play an important role in it.

M. L. Bøthun's study entitled "Time Course of Cerebrovascular Reactivity in Patients Treated for Unruptured Intracranial Aneurysms: A One-Year Transcranial Doppler

and Acetazolamide Follow-Up Study" addresses the time course of cerebrovascular reactivity (CVR) in patients treated for unruptured intracranial aneurysms, by comparing CVR within the first week after aneurysm treatment with CVR one year later. Factors that influence stability of CVR over time are also proposed in this study. The study of S. Lv et al. entitled "Compromised Dynamic Cerebral Autoregulation in Patients with Epilepsy" investigates cerebral autoregulation capability in patients with epilepsy during the interictal period and explores factors related to cerebral autoregulation parameters. The possible mechanisms of compromised cerebral autoregulation are discussed. N.-F. Chi et al. present a study entitled "Comparing Different Recording Lengths of Dynamic Cerebral Autoregulation: 5 versus 10 Minutes", in which dynamic cerebral autoregulation indices between 5- and 10-minute recordings in patients with ischemic stroke and healthy controls are compared. The study indicates that, to minimize the influences of time-dependent autoregulation variables, recording length should be unified in a single study or between studies.

The papers of this special issue provide new insights into cerebrovascular regulation in neurological disorders through its mechanisms, assessments, and clinical importance. Nevertheless, further studies are needed to help us to unlock the mystery of cerebrovascular regulation and its underlying mechanisms in neurological disorders, address technical challenges, and move on to providing the much-expected benefit to our patients.

Conflicts of Interest

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

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Bingren Hu
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Li Xiong

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Research Article

Influence of Induced Blood Pressure Variability on the Assessment of Cerebral Autoregulation in Patients after Cardiac Arrest

J. M. D. van den Brule,[1] **C. R. van Kaam**,[1] **J. G. van der Hoeven**,[1] **J. A. H. R. Claassen**,[2] **and C. W. E. Hoedemaekers**[1]

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Objective. To determine if increasing variability of blood pressure influences determination of cerebral autoregulation. *Methods*. A prospective observational study was performed at the ICU of a university hospital in the Netherlands. 13 comatose patients after cardiac arrest underwent baseline and intervention (tilting of bed) measurements. Mean flow velocity (MFV) in the middle cerebral artery and mean arterial pressure (MAP) were measured. Coefficient of variation (CV) was used as a standardized measure of dispersion in the time domain. In the frequency domain, coherence, gain, and phase were calculated in the very low and low frequency bands. *Results*. The CV of MAP was significantly higher during intervention compared to baseline. On individual level, coherence in the VLF band changed in 5 of 21 measurements from unreliable to reliable and in 6 of 21 measurements from reliable to unreliable. In the LF band 1 of 21 measurements changed from unreliable to reliable and 3 of 21 measurements from reliable to unreliable. Gain in the VLF and LF band was lower during intervention compared to baseline. *Conclusions*. For the ICU setting, more attention should be paid to the exact experimental protocol, since changes in experimental settings strongly influence results of estimation of cerebral autoregulation.

1. Introduction

Postanoxic encephalopathy is a common phenomenon after cardiac arrest and causes high mortality and morbidity [1]. Adapting the cerebral blood flow (CBF) to the cerebral metabolic demand improves cerebral recovery after cardiac arrest.

Cerebral autoregulation (CA) describes the process of cerebral vasodilation and vasoconstriction to maintain a stable CBF over a wide range of perfusion pressures. This adjustment of CBF to changes in cerebral perfusion pressure is directed by central regulation-mechanisms [2, 3] and by a local adaptation using myogenic vasoconstriction [4, 5]. In the low frequency band (LF, 0.07–0.2 Hz) blood pressure variations are provoked by sympathetic modulation of vascular tone [6]. Local vascular myogenic changes in blood pressure occur in the LF and very low frequency bands (VLF, 0.02–0.07 Hz) [7]. In the high frequency band (HF, 0.2–0.5 Hz) nitric oxide (NO) affects cardiovascular variability in animals [8]. Cerebral autoregulation is disturbed in a large proportion of patients after cardiac arrest [9, 10]. Disturbed autoregulation in the early phase after cardiac arrest is strongly associated with unfavourable outcome [9].

After cardiac arrest, the spontaneous variability of arterial pressure is significantly lower compared to age- and sex-matched control patients [11]. In addition, spontaneous variability in the mean flow velocity (MFV) in the middle cerebral artery as measured by transcranial Doppler (TCD) is also reduced. MFV variability restores towards normal values in survivors after cardiac arrest, while variability continues to decline in patients who do not survive [11].

For the estimation of dynamic CA, transfer function analysis (TFA) is considered the gold standard. TFA describes the dynamic relationship between blood pressure (input signal)

and CBF (output signal). This technique relies on spontaneous or induced fluctuations in MFV and mean arterial pressure (MAP). Reduced variability in the input signal will strongly reduce the reliability of the resulting output signal [12]. Recently, a white paper was published by the Cerebral Autoregulation Research Network (CARNet, http://www.car-net.org) with recommendations to improve the standardization and settings for TFA applications in studies of dynamic autoregulation [12]. Because of a lack of evidence in this matter, this paper could not formulate a recommendation regarding the minimum variability of the input signal that is required for reliable estimation of dynamic autoregulation. As a result, low variability may not be regarded as a criterion to reject or at least critically appraise CA data.

The main objective of our study was to determine if an increase in variability of the blood pressure signal influences determination of the state of autoregulation both in the time and in the frequency domain.

2. Materials and Methods

2.1. Study. A prospective observational study was performed at the intensive care unit (ICU) of a tertiary care university hospital in Nijmegen, the Netherlands.

2.2. Population. We studied 13 comatose patients admitted to the ICU after an out-of-hospital cardiac arrest. Inclusion criteria were age ≥ 18 years and a Glasgow Coma Score ≤ 8 after return of spontaneous circulation. Patients were included after written informed consent and approval of the protocol by the local Institutional Review Board (document number 2015-1567, 52259.091.15).

Exclusion criteria were an irregular heart rhythm, no transtemporal bone window, pregnancy, thrombolytic therapy, refractory cardiogenic shock, intra-aortic balloon pump, a life expectancy ≤ 24 hours and known carotid artery stenosis, or signs of carotid artery stenosis on physical examination or ultrasound.

2.3. Patient Management. The patients were treated according to the local protocol as described in a previous manuscript [11]. In short, this included mild therapeutic hypothermia 32–34°C for 24 hours, followed by passive rewarming to 37°C. All patients were sedated and sedation was stopped as soon as the temperature reached 36°C [11]. All patients were intubated and mechanically ventilated to obtain a PaO_2 > 75 mmHg and a $PaCO_2$ 34–41 mmHg [11]. An arterial catheter was used for monitoring of arterial blood pressure (ABP) and sampling of blood. According to this local protocol, MAP was maintained 80–100 mmHg [11].

2.4. Data Collection. Demographic, prehospital, and clinical data were collected. MFV in the middle cerebral artery was measured by TCD through the temporal window with a 2-Mhz probe (Multi-Dop T Digital, Compumedics DWL, Singen, Germany) as described in a previous manuscript [11]. All measurements were performed by two investigators (J.B. and C.H.). A 30-minute window of cerebral blood flow velocity (CBFV) and ABP was simultaneously recorded on a laptop computer and stored on a hard disk with a sample rate of 200 Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA).

2.5. Intervention. Baseline recordings of continuous ABP and CBFV were performed with the patient at rest, with the head of bed elevated at 30 degrees. The intervention consisted of repeated changes in the position of the bed from horizontal to maximum 30 degrees Trendelenburg and 30 degrees anti-Trendelenburg (period 6 minutes), thereby inducing low frequency blood pressure fluctuations. The blood pressure transducer was attached to the bed at right atrium level and its position changed simultaneously with the position of the patient. The patient was moved for 15 seconds from Trendelenburg to non-Trendelenburg and remained in this position for 45 seconds. A total of 3 Trendelenburg and 3 non-Trendelenburg positions were measured during the 6 minutes of testing. Changes were timed and recorded. During the measurements, PaO_2 and $PaCO_2$ were within normal ranges and stable.

Measurements were performed on admission to the ICU and at 24, 48, and 72 hours.

2.6. Data Analysis. CBFV and ABP data were analyzed using custom-written MATLAB scripts (Matlab R2014b, The MathWorks Inc., Massachusetts, USA), as described in a previous manuscript [11]. From these ABP and CBFV signals, 5-minute segments of baseline and intervention data were selected based on the least amount of artefacts [11]. MAP and MFV were acquired by filtering ABP and CBFV with a third-order zero phase-lag Butterworth filter with a cut-off frequency of 0.5 Hz [11]. By averaging these 5-minute windows of the MAP and MFV signals, mean values of MAP and MFV were acquired [11]. For transfer function analysis, the CARNet TFA MATLAB script was used (available on http://www.car-net.org).

To validate our analysis, an external expert (J.C.) went through the analysis step by step to check whether this analysis had been performed in agreement with the recommendations in the white paper on transfer function analysis of the International Cerebral Autoregulation Research Network [12].

2.7. Blood Pressure Variation. Coefficient of variation (CV) was used as a standardized measure of dispersion for both MAP and MFV in the time domain. CV was defined as the standard deviation of the signal divided by the mean of the signal and was calculated from all filtered signals. This way, the variation is expressed in percentage of the mean.

In the frequency domain, the average spectral power of MAP and MFV was calculated as a measure of variation in the very low (VLF, 0.02–0.07 Hz) and low (LF, 0.07–0.2 Hz) frequency bands.

2.8. Cerebral Autoregulation. CA was calculated in the time domain and frequency domain. In the time domain, CA was calculated by the mean flow velocity index (Mx) as a Pearson's correlation coefficient between 10-second averages of ABP and CBFV over the 5-minute time window [13]. A cut-off

Table 1: Clinical and laboratory data of cardiac arrest patients on admission. PEA: pulseless electrical activity; VF: ventricular fibrillation; VT: ventricular tachycardia.

Characteristics	
Age (years)	61 [50–65]
Male (*n*, %)	12/13 (92%)
Initial rhythm VT/VF (*n*, %)	12/13 (92%)
Initial rhythm PEA (*n*, %)	1/13 (8%)
SAPS 2	58 [42–77]
APACHE 2	27 [18–30]
pH	7.01 [6.94–7.27]
Lactate (mmol/L)	8.5 [7.0–13.3]
PaCO2 (mmHg)	7.0 [6.2–9.5]

value of 0.3 was chosen to indicate absence or presence of autoregulation [14].

In the frequency domain, TFA was performed according to the recommendations by the international Cerebral Autoregulation Research Network (CARNet) [12]. In short, the 5-minute segments of MFV and MAP signals were resampled to 10 Hz. Both auto- and cross-spectra were estimated based on Welch's method using a sliding Hanning window of 100 seconds' window length with 50% overlap. Spectral smoothing was applied by using a triangular moving average window. Gain and phase were only calculated when coherence was above the critical coherence threshold based on the 95% confidence interval. The cut-off value was 0.34. The TFA coherence, gain, and phase were calculated for the very low (VLF, 0.02–0.07 Hz) and low (LF, 0.07–0.2 Hz) frequency bands.

2.9. Statistical Analysis. Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). Data were checked for Gaussian distribution by a Kolmogorov-Smirnov test. Paired, normal distributed data were analyzed with a paired *t*-test and presented as mean with standard deviation. Paired non-Gaussian distributed data were analyzed with a Wilcoxon matched-pairs signed rank test and presented as median with 25th and 75th percentile.

A p value of <0.05 was considered to indicate significance.

3. Results

3.1. Demographic and Clinical Data. We included 13 comatose patients successfully resuscitated after cardiac arrest and treated with mild therapeutic hypothermia. The median age was 61 [50–65] years. Twelve patients had ventricular fibrillation (VF) or ventricular tachycardia (VT) as initial rhythm, and 1 patient initially had a pulseless electrical activity (PEA). Four patients died in the ICU, all because of severe postanoxic brain damage. The clinical and laboratory data on admission are summarized in Table 1.

3.2. Mean Arterial Blood Pressure Variation and Mean Flow Velocity Variation in the Time Domain. Tilting of the bed induced a significant increase in CV of MAP from 3.056 ± 1.464 at baseline to 8.238 ± 2.646 during intervention ($p < 0.0001$) (Figure 1). The CV of MFV after cardiac arrest was also significantly higher during the intervention (7.571 [6.124–9.176]) compared to baseline (6.090 ± 3.613) ($p = 0.0028$) (Figure 1).

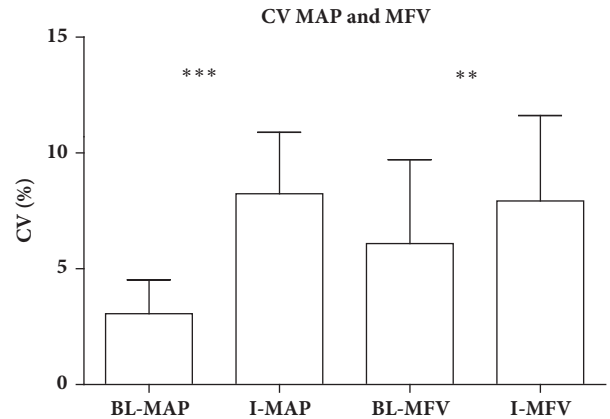

Figure 1: CV of MAP and MVF after cardiac arrest in baseline position and during intervention. BL: baseline; CV: coefficient of variation; I: intervention; MAP: mean arterial pressure; MVF: mean flow velocity.

3.3. Cerebral Autoregulation

3.3.1. Time Domain. On group level, Mx did not change during intervention (0.5669 [0.0132–0.7712]) compared to baseline (0.3757 ± 0.4473) ($p = 0.6776$) (Figure 2). On individual level, in 9 of the 21 measurements (43%) interpretation of the CA changed during intervention. Five measurements changed from intact to impaired CA and 4 measurements changed from impaired to intact CA.

3.3.2. Frequency Domain. There were no significant changes on a group level in coherence between baseline (0.4449 ± 0.2692) and intervention (0.4313 ± 0.2702) in the VLF band ($p = 0.8281$) (Figure 3). There were also no changes in coherence in the LF band between baseline (0.3476 [0.2655–0.6949]) and intervention (0.3043 [0.2361–0.6444]) ($p = 0.3265$) (Figure 4). On an individual level, in the VLF band, 5 of the 21 measurements changed from unreliable to reliable and 6 of the 21 measurements changed from reliable to unreliable. In the LF band 1 of the 21 measurements changed

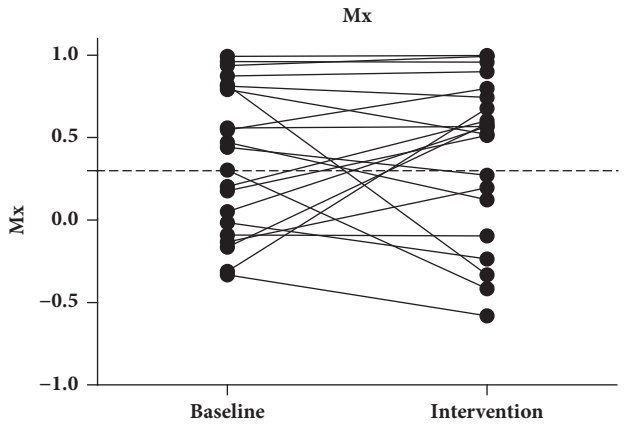

Figure 2: Mx after cardiac arrest in baseline position and during intervention. Five of the 21 measurements changed from intact to affected CA and 4 of the 21 measurements changed from affected to intact CA. Mx: mean flow velocity index.

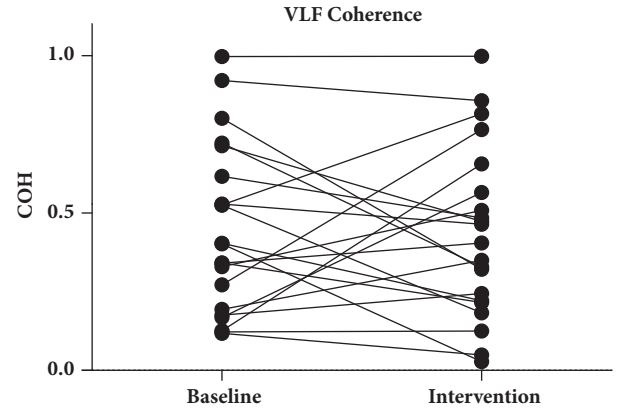

Figure 3: Coherence in the VLF band. Five of the 21 measurements changed from unreliable to reliable and 6 of the 21 measurements changed from reliable to unreliable. VLF: very low frequency.

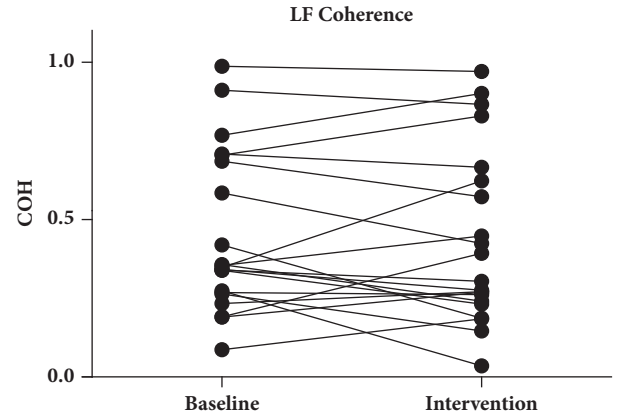

Figure 4: Coherence in the LF band. One of the 21 measurements changed from unreliable to reliable and 3 of the 21 measurements changed from reliable to unreliable. LF: low frequency.

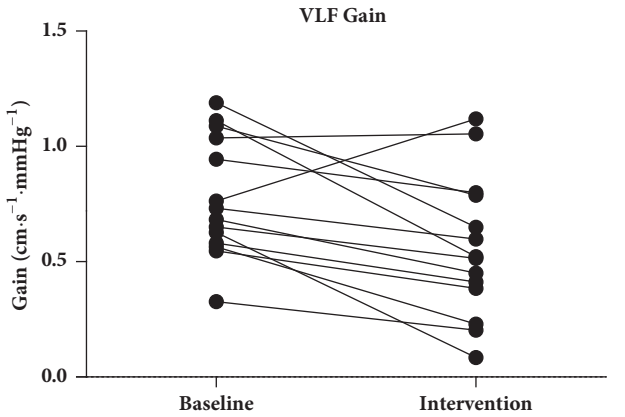

Figure 5: The gain in the VLF band during intervention was lower compared to the gain in the VLF baseline ($p = 0.0058$). VLF: very low frequency.

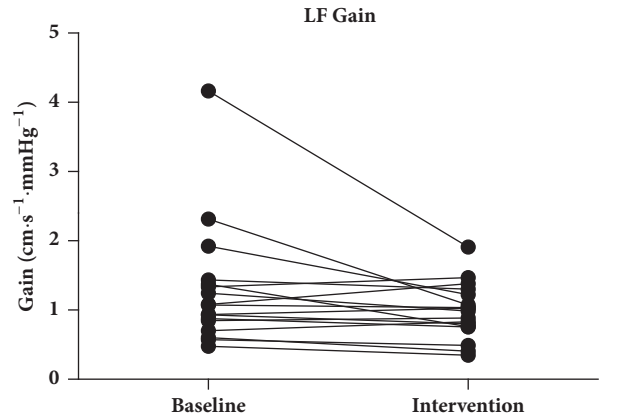

Figure 6: The gain in the LF band during intervention was lower compared to the gain in the LF band baseline ($p = 0.0523$). LF: low frequency.

from unreliable to reliable and 3 of the 21 measurements changed from reliable to unreliable.

The gain in the VLF band during intervention (0.5578 ± 0.3046) was lower compared to the gain in the VLF baseline (0.7746 ± 0.2579) ($p = 0.0058$) (Figure 5). The gain in the LF band during intervention (0.9817 ± 0.4007) was lower compared to the gain in the LF band baseline (1.075 [0.7757–1.405]) ($p = 0.0523$) (Figure 6).

There were no changes in phase in the VLF and LF band during intervention (21.29 [11.73–38.64] and 14.96 [−3.546–27.16], respectively) compared to the phase in baseline position (36.12 ± 31.01 and 12.00 [0.1032–22.62], respectively) ($p = 0.4548$ and 0.570, respectively) (Figure 7).

4. Discussion

Amplification of blood pressure variability changed the interpretation of CA as measured by Mx and TFA. This difference in test results is not related to physiological changes in CA, as both baseline and intervention were performed at the same time-point after cardiac arrest. This difference in test results

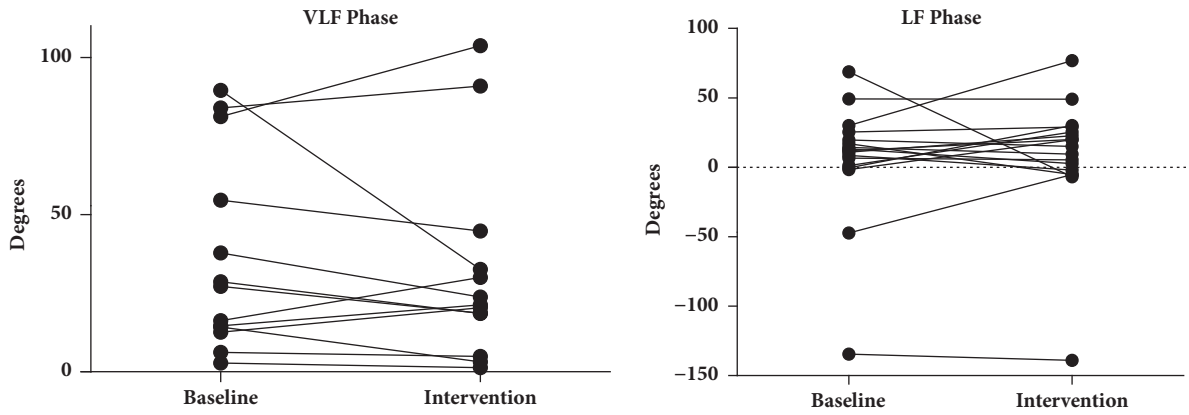

FIGURE 7: There were no changes in phase in the VLF and LF band during intervention compared to the phase in baseline position ($p = 0.4548$ and 0.570, respectively). LF: low frequency; VLF: very low frequency.

is most likely related to bias induced by very low variability in the blood pressure and MFV signal at baseline, which renders estimation of CA with these methods unreliable.

For the estimation of dynamic CA, TFA is considered the gold standard. The white paper on TFA methodology provides recommendations on the optimal cut-off values of coherence and parameter settings [12]. No recommendations are available for the optimal experimental or clinical protocol. High quality of both the ABP and MFV signal are essential for reliable estimation of CA [15]. Poor quality of the temporal bone insonation conditions or poor quality of the arterial or MFV signal can result in considerable bias of the TFA results. Quality of both signals was adequate in our study and did not explain the intervention induced changes in TFA and Mx in our population.

The optimal recording time of signals for autoregulation to stabilize is largely unknown. Recording time for the autoregulatory index Mx significantly influences validity of the measurements. Mx calculated on intervals shorter than 6 minutes is at risk for changes due to insufficient stabilization of the signal [16]. The Mx data were determined from a 5-minute time window, possibly influencing the validity of the Mx.

The white paper recommends a minimum window length for TFA analysis of at least 100 seconds [12]. This is based on the fact that window lengths shorter than 75 seconds resulted in increased bias [12]. Recording time of the TFA signals at baseline and during intervention in our study was sufficiently long to avoid bias related to time restriction.

The extent to which TFA outcomes are influenced by quality of the signals differs between different parameters. In our study, increased variability of signals resulted in decrease in gain in both the LF and VLF bands, whereas phase remained stable. This is most likely related to the fact that gain reflects the strength of the autoregulation, which may be changed by increased amplitude of the signal. In contrast, phase is related to velocity of dynamic changes in autoregulation. Since augmentation of the variability is unlikely to change the timing of the arterial and cerebral pulses, phase may remain unchanged during the intervention.

The use of the coherence function is recommended to identify conditions where estimates of gain and phase may not be reliable. The intervention changed the level of coherence from unreliable to reliable in a substantial portion of patients, but the reverse (change from reliable to unreliable) occurred in a similar proportion of patients. Since the variability was extremely low, the input signal may have been too low for adequate determination of TFA coherence at baseline.

This was supported by visual inspection of the raw data and linking this with individual outcomes of TFA. Visual inspection of the raw signals can easily identify whether oscillations in MAP and CBFV are present and whether these oscillations are temporally related. In the few patients who showed variability in both MAP and CBFV, the results of TFA appeared reliable, with higher phase values in VLF than in LF and lower gain values in VLF than in LF. In contrast, when TFA was performed in patients with low MAP variability, these normal patterns were not observed, even if coherence was above the threshold.

In an earlier study we proved that the spontaneous variability of the MAP remained low during the entire study period after cardiac arrest [11]. In the current study we demonstrated a significant increase in CV of MAP during tilting of the bed compared to the resting position. Previous literature showed an improvement of reproducibility of the autoregulation coefficients by inducing blood pressure variability due to a sit-to-stand manoeuvre [17, 18]. However, those manoeuvres lead to consistent oscillatory changes that enhance reliability, in contrast with bed tilting.

This study has a number of limitations. We performed an observational study in a relatively small population. Tilting of the bed may result in changes in quality of the recorded signal, such as loss of signal, motion artefacts, and baseline drifts. Reanalysis of the raw signals however was carefully performed by an independent expert (J.C.), using only good quality signals and verifying correct TFA settings, but this did not alter the outcome. Changes in the bed position may influence the pressure in the veins draining the cerebral venous blood. These changes in venous outflow pressure

may influence cerebral perfusion and thus the validity of the autoregulation measurements.

5. Conclusions

The white paper on TFA methodology currently provides no recommendations about minimal necessary blood pressure variation to perform a reliable TFA. Given the results of this study, addition of minimal necessary variation of input signals to the white paper on TFA methodology may improve the validity of TFA measurements. For the ICU setting, more attention should be paid to the exact experimental protocol, since changes in experimental settings strongly influence results of estimation of CA. The optimal level of variation of the signals will be difficult to establish because of the lack of golden standards of autoregulation for comparison of the results. Computer modelling may be helpful to establish the effects of changes in different parameters on the measurements of autoregulation.

Abbreviations

ABP: Arterial blood pressure
CA: Cerebral autoregulation
CBF: Cerebral blood flow
CBFV: Cerebral flow velocity
CV: Coefficient of variation
ICU: Intensive care unit
MAP: Mean arterial pressure
Mx: Mean flow velocity index
NO: Nitric oxide
PEA: Pulseless electrical activity
TCD: Transcranial Doppler
TFA: Transfer function analysis
VF: Ventricular fibrillation
VT: Ventricular tachycardia.

Conflicts of Interest

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

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Review Article

Futile Recanalization after Endovascular Therapy in Acute Ischemic Stroke

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Early recanalization after endovascular treatment could improve the prognosis of acute ischemia stroke. Futile recanalization often occurred which was one of the main causes of failure. By now the mechanisms of futile recanalization were not clear. They are probably concerned with bad collateral circulation, subacute reocclusion, large hypoperfusion volumes, microvascular compromise, and impaired cerebral autoregulation. Previous research found that some of the image markers could be used as the accurate predictors for poor prognosis after successful treatment in order to identify the patients who were not suitable for recanalization and reduce some of the unnecessary cost. Predictors for futile recanalization mentioned in our article can be used for supplement to make decision for endovascular treatment.

1. Introduction

It has been proved that early recanalization could reduce mortality of acute ischemic stroke patients with large vessel occlusion and improved the prognosis finally. Five randomized controlled trials proved the efficacy of endovascular treatment over standard medical care in patients with acute ischemic stroke caused by large vessel occlusion of the proximal anterior circulation [1–4]. For many interventional radiologists, the main goal of endovascular treatment is to complete recanalization. However, it was upsetting sometimes that not all patients had good clinical outcome at the end despite a perfect revascularization. It often occurs when successful recanalization fails to bring favorable prognosis which called futile recanalization. Studies with first-generation IA thrombectomy device found that the rate of futile recanalization after endovascular treatment was over 60% which had not improved outcomes compared with intravenous thrombolysis alone [5]. It was still a challenge when determination was made by neurologist or interventional radiologist after a great success of second-generation IA thrombectomy devices. A meta-analysis of individual patient data from the trials with the MR CLEAN, EXTEND-IA, ESCAPE, SWIFT PRIME, and REVASCAT found that the rate of futile recanalization after endovascular treatment was 54% [6]. In this review, we will highlight the predictors for poor outcome following endovascular treatment.

We performed a major online database search including Medline, PubMed, Cochrane Library, and EMBASE to identify any related articles using the key words "futile recanalization". There was not a clear definition of futile recanalization. Some of pervious papers summarized it as poor clinical outcomes after an adequate vessel recanalization for patients with acute stroke [7]. In another study, "futile recanalization" was defined by the occurrence of poor functional outcome (mRS score larger than 3 at 1–3 months) despite complete angiographic recanalization (TIMI grade 2b or 3) [5].

2. Causes and Mechanism for Futile Recanalization

2.1. Futile Recanalization and Cerebral Blood Flow Regulation. Previous research had studied the correlation between

cerebral blood flow regulation and outcomes after using recombinant tissue plasminogen activator (rtPA) for acute cerebral infarction [8]. Previous publications on cerebral autoregulation in ischemic stroke found a transient impairment of cerebral autoregulation during the subacute stages of sever cerebral infarction [9–11]. Surprisingly, animal study showed that rtPA displays neurotoxic properties. According to the results of this study, the use of rtPA might destroy the blood-cerebral barrier, damage blood vessels, and impair the cerebral autoregulation possibly [12]. But the outcome of humans study was opposite which concluded that the use of rtPA did not conduce to impaired cerebral autoregulation. However, cerebral autoregulation after rtPA treatment in this study was evaluated 10–20 h later, so it was possible to ignore the initial detrimental effect by rtPA on cerebral autoregulation [8]. Some researchers believe that impaired cerebral autoregulation may be one of the possible mechanisms of futile recanalization. The mechanisms of cerebral blood flow regulation during and after intravenous thrombolysis are not clear, not to mention the mechanical recanalization. More research focused on the evaluation of cerebral autoregulation influenced by endovascular treatment is needed to explore the correlation with futile recanalization.

2.2. Futile Recanalization and Hypoperfusion Volume. The existence and the range of ischemic penumbra which is salvageable potentially can conceivably change the prognosis. Hypoperfusion volumes could indicate final infarct volume [13]. It was observed in over 40% of patients after intra-arterial thrombolysis recanalization a few hours later [5]. It has been demonstrated that most hypoperfused tissue developed into real infarction in seven days. Initial hypoperfusion was linked to selective neuronal loss in rescued penumbra probably [14]. The patients with large hypoperfusion volumes were more likely to have poor prognosis.

2.3. Futile Recanalization and Collateral. The prognosis could be influenced by collateral circulation which made tissue viability sustain until effective recanalization [15]. Proximal occlusion usually affected larger areas of brain tissue and often came to poor outcome finally [16]. Reocclusion after recanalization often occurs immediately [17], hours later, or in the first 24 hours. Previous research found that reocclusion was associated with neurologic deterioration which occurred in nearly 10% of patients receiving intra-arterial thrombolysis [18–20].

2.4. Futile Recanalization and Microvascular Compromise. Another important factor is microvascular compromise which could influence effective tissue perfusion despite macrovascular recanalization at the capillary level. It occurs after leukocytes and platelets aggregation causing plugging of microvessels due to endothelial activation. These changes of microcirculation disturbance have been associated with unfavourable prognosis following percutaneous transluminal coronary angioplasty (PTCA) after the first attack of AMI [21]. This issue about acute stroke has not been studied up to now.

2.5. Technology Difference between IA Thrombectomy Devices. As we mentioned before, the use of the second-generation IA thrombectomy device such as Solitaire improved the prognosis compared with the first-generation IA thrombectomy device such as Merci. The occurrence rate for FR was a little decreased. It is believed that technical progress of method of endovascular treatment may bring about new changes. But until now the occurrence rate for FR was still high, although with these new thrombectomy device we could improve the prognosis finally. And most of the time the mechanism for occurrence of futile recanalization was similar. The exact physiopathologic mechanisms of different thrombectomy device are unclarified yet.

By now the mechanisms of futile recanalization were not clear. They are probably concerned with bad collateral circulation, subacute reocclusion, large hypoperfusion volumes, microvascular compromise, and impaired cerebral autoregulation.

3. Prediction for Futile Recanalization

Severe cerebrovascular disease not only brings body's illness and the life pressure to patients, but also brings serious economic burden to patients' family. The aim of reperfusion therapy in acute ischemia stroke as we expect is not only to recanalize the occluded vessel but also to save the ischemic but still viable brain tissue. It could promote the prognosis of most patients with acute stroke which is the most effective treatment at present. However, these treatments are often expensive and are often not available on Social Security and Medicare. Some of the families can hardly afford the cost of such bridging treatment. The reliable prediction of futile recanalization is important which can identify the patients who are not suitable for recanalization and reduce some of the unnecessary cost.

3.1. Clinical Features. In a multicenter study [5], individual data of acute ischemic stroke treated with endovascular treatment combined from six studies were analyzed. 96 of 270 patients after intra-arterial thrombolysis achieved complete recanalization. It had been observed that 47 patients (49%) satisfied the definition of futile recanalization. High baseline NIHSS score (NIHSS score > 10), older age (age > 70 years), and longer delay have been identified as possible predictors for poor outcome after complete recanalization [22]. This is the first article focused on futile recanalization, especially among elderly and severe patients. These findings had been confirmed by following studies. But the sensitivity of these factors mentioned was not high enough. And the data of six studies had obvious heterogeneity and some of them did not use the new technique of endovascular therapy. Reliable makers need to be established.

Some clinical features could be used for early discrimination for futile recanalization. High baseline NIHSS score was associated with FR, which was mentioned in most of the research about FR, although some research found that the patients with high NIHSS score could be benefit from endovascular treatment. But there is no avoiding the fact that high baseline NIHSS score is more likely for the occurrence

of FR [23–25]. It was a controversial issue about age and many doctors hold quite different opinions. Available data suggested that patients with older age easily developed FR [5, 25]. Another clinical features mentioned more frequently included longer delay and ischemic lesion which might be associated with FR in some research. Although the prognosis for endovascular treatment was different between anterior circulation and posterior circulation, it was unexpected that there was not any clear correlation between vascular territory for FR in these studies [5, 25, 26]. We think it could be because the poor prognosis for stroke after ET depends not only on the occurrence of FR, but equally on other factors. Delay from missed diagnosis, technical difficulty, and the recanalization rate may be possible cause. Therefore, high baseline NIHSS score, older age, and longer delay have been identified as possible predictors for poor outcome after complete recanalization.

3.2. Imaging Markers. Owing to our research of recent articles about prediction of recanalization, it was found that some of the image markers can be used as the accurate predictors for poor prognosis after successful treatment. The recently published studies attempted to use some imaging criteria for selection of patients for mechanical thrombectomy to improve the odds of good outcomes.

3.2.1. Large DWI-DWM Lesion. Tateishi and his team [27] found that large ischemic lesions in the deep white matter (DWM) on pretreatment diffusion-weighted MRI (DWI) might be a probable predictor for futile recanalization. The author defined large DWI-DWM lesion as a hyperintense lesion in the DWM on first DWI, located mainly between the anterior and posterior horns of the lateral ventricle. In 35 of 46 consecutive patients (76%) with complete recanalization, 20 patients after successful recanalization had a poor prognosis finally. Higher baseline NIHSS scores and older age could predict futile recanalization which was consistent with previous research; the study also found that a higher prevalence of large DWI-DWM lesions is associated with futile recanalization (45 versus 9%; $p = 0.022$). The positive predictive value for utile recanalization was nearly 90%. Patients with large preintervention DWI-DWM lesions may be poor candidates for endovascular therapy. However, the author also found that ASPECTS on CT and on DWI and initial ischemic lesion volume on DWI were not confirmed to have apparent correlation with futile recanalization which was inconsistent with other studies. It was probably due to small sample size and some of them did not use the new technique of endovascular therapy. Similarly, the usefulness of DWI-DWM needs to be confirmed.

3.2.2. Leukoaraiosis. Leukoaraiosis (LA) is a radiological phenomenon that represents white matter lesions in the brain. Prior research indicated that hypoperfusion ischemia is probably the main cause for white matter abnormalities. The hypothesis is that structural and functional microvascular abnormalities in patients with LA have been supported by studies before. The brain tolerance from ischemic might be reduced when moderate-severe LA was diagnosed. It was unpromising to make the decision for recanalization procedures with patients with moderate-severe LA, because it often indicates poor microvascular cerebral reserve so that after vessels occlusion irreversible brain infarct rapidly develops [28, 29]. A recent study conducted by Gilberti and her teammates [24] was aimed at evaluating whether LA could predict futile recanalization in patients with anterior circulation LVO. 68 patients treated with endovascular therapy and achieving complete recanalization were included: 22 patients of them had a poor prognosis. By using of multivariable analysis, they found that moderate-severe LA was an independent predictor of FR ($p = 0.01$).

3.2.3. ADC Quantification of Ischemic Lesions at Baseline MRI. In a small series [30], the researcher proved the prognostic value of baseline ADC quantification in patients with BAO undergoing EVT. 11 patients with BAO undergoing EVT were retrospectively investigated. They found that the lower values of minimum ADC at admission MRI are strongly correlated with higher scores in mRS at discharge ($p = 0.009$). And there was a negative correlation between minimum ADC and NIHSS at admission ($p = 0.02$), mRS at three months, and difference between pre- and posttreatment ischemic area ($p = 0.026$). Ischemic area and TICI grade were not significantly associated with clinical results. ADC quantification of ischemic lesions at baseline MRI seems to predict clinical outcome in patients with BAO undergoing EVT, more importantly than ischemic area or TICI grade.

3.2.4. ASPECTS on CT Angiography Source Images. The recently published studies found that the appropriate selection of patients for mechanical thrombectomy upon imaging criteria helps improve prognosis. The Alberta Stroke Program Early CT Score (ASPECTS) [31] from initial noncontrast CT (NCCT) could predict FR which the pervious study mentioned [32, 33]. But the reliability of this score was low. Its usefulness for decisions about thrombolytic therapy has been debated, and a lower baseline ASPECTS score was one of the exclusion criteria for most recent trials of MT [34]. ASPECTS on CT angiography source images (CTA-SI-ASPECTS) was a more reliable indicator of outcome and final infarct volume in acute ischemic stroke [35, 36]. 110 patients with acute stroke from the FUN-TPA study registry [37] were included. All of the patients included had anterior circulation LVO and received reperfusion therapies. Kawiorski and his teammates attempted to assess whether the baseline of this score might help predict response to treatment and futile recanalization after reperfusion therapies reliably [25]. Total recanalization rate was 71%; 28% of cases were futile recanalization. Initial CTA-SI-ASPECTS was correlated with futile recanalization (OR 0.5; 95% CI 0.3–0.7); however NCCT-ASPECTS was not correlated with futile recanalization (OR 0.8; 95% CI 0.5–1.2). The score of CTA-SI-ASPECTS less than five was the optimum cut-off for prediction of futile recanalization (positive predictive value 86%; negative predictive value 77%; sensitivity 35%; specificity 97%). CTA-SI-ASPECTS is a reliable predictor for futile recanalization and could be used for treatment decisions for revascularization therapies.

3.2.5. Collateral. A recent study [38] investigated whether the evaluation of collateral can be used as prediction for futile recanalization in acute ischemia stroke after recanalization. Collateral plays an important role in the pathophysiology of acute ischemic stroke and is identified as a conceivable predictor of FR. 135 anterior circulation stroke patients who received intravenous thrombolysis were retrospectively analyzed in this research. They finally put forward an equation using their collateral score (adjusting for baseline NIHSS, age, and recanalization) which emerged as a statistically significant prognostic biomarker for good prognosis ($p < 0.033$) among patients after completed recanalization, but not appropriate for nonrecanalized patients ($p < 0.497$). The results showed that collateral score was a reliable marker for prediction ($p < 0.044$). The poor collaterals predict poor prognosis despite successful recanalization and robust collaterals warrant consideration for recanalization therapy promoting the chance of better prognosis. Some other studies confirmed these conclusions in patients after ET.

3.2.6. Combined Score on Multimodal Computed Tomography. Another study [23] aimed to assess the accuracy of parameters on multimodal computed tomography (CT) we mentioned before. The author also tried to use their combination for predicting futile recanalization after endovascular treatment. They retrospectively analyzed the data of a cohort of consecutive patients with stroke of anterior circulation. 57% of the patients among 150 patients included had futile recanalization. They accessed the predictive ability of ASPECTS on nonenhanced CT, CT angiography source images, CBF, CBV, mismatch CBV–CBF, and poor collaterals for futile recanalization. Among these parameters, ASPECTS on CT angiography source images ≤ 5, ASPECTS on CBV ≤ 6, and poor collaterals could predict futile recanalization independently after multivariate analyses. The combined score consisted of these parameters and could provide much more information: 57% of the patients with score 1, 89% with score 2, and 100% with score 3 had futile recanalization. Reclassification analyses indicated that the combined multimodal CT score predicted futile recanalization reliably. The prognostic value of this score needs more large studies to confirm.

Successful treatment of stroke patients especially with occluded vessel requires rapid and effective reperfusion therapies, which include mechanical thrombectomy and other endovascular treatment. One of the keys to success is the proper selection of patients in order to perform not only successful recanalization but also successful recovery. Treatment according to standards in current guideline was not enough. Futile recanalization was unexpected and not uncommon. Some of the indicators for futile recanalization in the past studies have been mentioned in this article which could be used for supplement for the clinician to make suitable decision. Further research is needed to understand the mechanism of futile recanalization and the correlation with cerebral blood flow regulation.

Disclosure

Ximing Nie is the first author.

Conflicts of Interest

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

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Review Article

Cerebral Perfusion and Cerebral Autoregulation after Cardiac Arrest

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Out of hospital cardiac arrest is the leading cause of death in industrialized countries. Recovery of hemodynamics does not necessarily lead to recovery of cerebral perfusion. The neurological injury induced by a circulatory arrest mainly determines the prognosis of patients after cardiac arrest and rates of survival with a favourable neurological outcome are low. This review focuses on the temporal course of cerebral perfusion and changes in cerebral autoregulation after out of hospital cardiac arrest. In the early phase after cardiac arrest, patients have a low cerebral blood flow that gradually restores towards normal values during the first 72 hours after cardiac arrest. Whether modification of the cerebral blood flow after return of spontaneous circulation impacts patient outcome remains to be determined.

1. Introduction

The prognosis of cardiac arrest patients is mainly determined by the extent of neurological injury induced by the circulatory arrest. Return of spontaneous circulation (ROSC) does not naturally result in recovery of cerebral perfusion, as cerebral perfusion failure after ROSC is well described in animal models with no-reflow, hypoperfusion, and hyperperfusion. In animals, cerebral blood flow (CBF) ultimately restores towards normal [1]. Human studies have revealed that, in the early phase after cardiac arrest, patients have a low CBF that gradually restores towards normal values during the first 72 hours following the arrest [2–4]. In the first part of this review, the temporal course of cerebral blood flow after ROSC is described. This is relevant, because changes in cerebral blood flow can contribute to secondary brain injury. The second part of this review will focus on cerebral autoregulation after cardiac arrest, because this is an important factor in the development of ischaemia and secondary brain damage.

2. Cerebral Blood Flow after Cardiac Arrest

Cerebral perfusion after resuscitation is characterized by early hyperemia followed by hypoperfusion and, finally, restoration of normal blood flow. Furthermore, the blood flow is heterogeneous, with areas of no flow, low flow, and increased flow at the level of the microcirculation [5].

2.1. Early Hyperemia (Vasoparalysis) (0–20 min after ROSC). Reduction of vascular tone due to tissue acidosis leads to vasoparalysis [6], which does not respond to changes in blood pressure or CO_2 [7]. Hypoxia-induced vasoparalysis has been demonstrated in rats in the very early phase of cardiac arrest [8] and is suggested to result from an imbalance between vasodilatory and vasoconstrictive mediators in the cerebral circulation, including nitric oxide (NO) [9] and adenosine [10]. There is no direct evidence for this phenomenon *in vivo*. Hyperemia, in combination with brain swelling, can cause increased intracranial pressure, which usually normalizes before the hypoperfusion phase initiates [11]. Antioxidants and polynitroxyl albumin represent therapies that may be of value in the early hyperemia phase [12, 13].

2.2. Hypoperfusion Phase (20 min–12 h after ROSC). The hypoperfusion phase is due to an impairment of the metabolic/hemodynamic coupling mechanisms, and its severity is independent of the duration of ischaemia [14]. We confirmed this lack of a relationship between ischaemia duration and the

severity of hypoperfusion in comatose patients after cardiac arrest (data not published) [15]. During the hypoperfusion phase, the CBF decreases by approximately 50% [16, 17]. Several factors are implicated to play a role, including endothelial damage and an imbalance of local vasodilators (NO) and vasoconstrictors such as endothelin [18]. In this phase, impairment of the autoregulation may further decrease CBF in the setting of low blood pressure. Viable therapies for this hypoperfusion phase have been examined in animal models and include 20-hydroxyeicosatetraenoic acid inhibition by HET0016, nimodipine, and endothelin type A-antagonists [19–23].

2.3. Restoration of Normal Blood Flow (12–72 h after ROSC). Finally, CBF returns to normal, remains low, or increases [24, 25]. In more recent literature, only a return to normal or an increase in CBF is described [2, 26]. Bisschops et al. described a low mean flow velocity in the middle cerebral artery (MFV_{MCA}) on admission, which remained relatively stable during the first day and increased to normal levels at 48 hours [2].

The MFV_{MCA} was shown to be similar in survivors and nonsurvivors upon ICU admission [26]. However, in survivors of cardiac arrest, the MFV_{MCA} increases towards normal values in the following 72 hours, whereas a much more pronounced increase in MFV_{MCA}, resulting in an overshoot of CBF, was observed in nonsurvivors [26]. This overshoot is most likely the result of a loss in vascular tone resulting in a decrease in cerebrovascular resistance in these nonsurvivors [26].

Low CBF after cardiac arrest may cause a mismatch between cerebral oxygen demand and supply. A reduction in cerebral metabolism after cardiac arrest has been described in humans and animals [27–31]. In the first 48 hours after cardiac arrest, cerebral oxygen extraction remains normal with a low CBF. This low CBF is not associated with anaerobic metabolism, determined by the jugular venous-to-arterial CO_2/arterial-to-jugular venous O_2 content difference ratio [32]. The jugular venous CO_2 content significantly decreases after cardiac arrest, suggestive of low CO_2 production due to low cerebral metabolism [32].

In survivors, the MFV_{MCA} is low immediately after cardiac arrest, accompanied by low metabolism, with a gradual restoration towards normal values accompanied by restoration of metabolism. This gradual increase of metabolism in survivors is consistent with recovery of neuronal activity. These results imply that the cerebrovascular coupling is intact in patients with a favourable neurological outcome.

In contrast, in nonsurvivors with cerebral hyperfusion, the cerebral oxygen extraction is strongly reduced, suggesting decoupling of cerebral flow and metabolism in nonsurvivors. This ongoing low metabolism likely reflects irreversible neuronal damage [32].

3. Cerebral Autoregulation following Cardiac Arrest

3.1. Cerebral Autoregulation. Generally, it is assumed that cerebral autoregulation maintains CBF at a constant level when the mean arterial pressure (MAP) is between approximately 50 and 150 mmHg (the plateau phase) (Figure 1). However, more recent data suggest that cerebral autoregulation maintains constant blood flow in a smaller range [33, 34] (Figure 2). Cerebral autoregulation is more effective in the range above baseline MAP than below baseline MAP [35] (Figure 2).

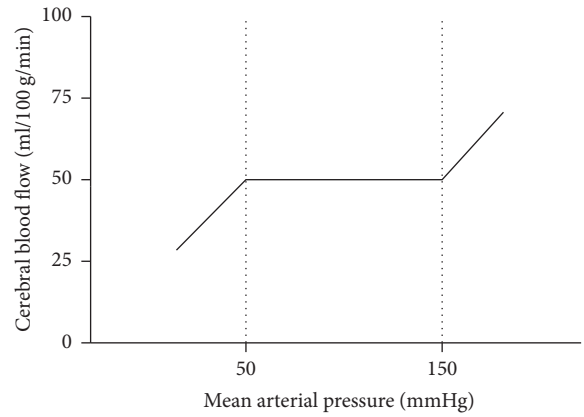

Figure 1: Cerebral autoregulation maintains cerebral blood flow at a constant level when the mean arterial pressure is between approximately 50 and 150 mmHg (the plateau phase).

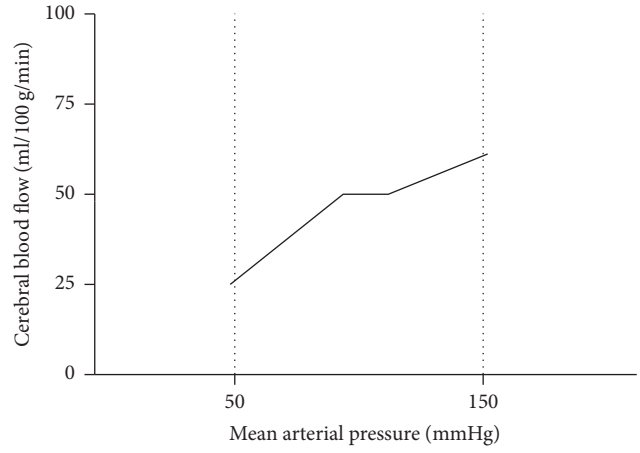

Figure 2: More recent data support the opinion that cerebral autoregulation does not maintain constant blood flow through a broad MAP range of 50–150 mmHg, but probably in a smaller range. Cerebral autoregulation is more effective in the range above baseline mean arterial pressure, compared to the range below.

The upper and lower limits of cerebral autoregulation are not fixed [36]. For example, chronic hypertension shifts these limits up. This adaptation protects the brain against high blood pressure but makes it also more vulnerable to hypoperfusion during periods of hypotension.

Dynamic cerebral autoregulation is clinically more relevant than static autoregulation, because it protects the brain against rapid alterations in blood pressure. Various methods and models are available for estimating dynamic cerebral autoregulation, using both spontaneous and induced fluctuations in blood pressure.

3.2. Cerebral Autoregulation following Cardiac Arrest. Cerebral autoregulation after cardiac arrest has been investigated in various studies. Initially, a linear relationship was demonstrated between MAP and CBF [37], suggesting a completely dysfunctional (static) cerebral autoregulation after cardiac arrest. Static cerebral autoregulation curves were constructed for patients after cardiac arrest by stepwise increasing MAP with vasopressors and simultaneous determination of CBF using TCD [38]. Of the 18 patients after cardiac arrest studied by Sundgreen et al., static cerebral autoregulation was absent in 8 and present in 10 patients. In five out of ten patients with preserved cerebral autoregulation, the lower limit of autoregulation was shifted upwards (range 80–120 mmHg) [38]. In fact, autoregulation may remain intact, but with a narrowed and upward shifted intact zone. This study demonstrated the heterogeneous nature of cerebral autoregulation in cardiac arrest patients.

Ameloot et al. showed that (dynamic) cerebrovascular autoregulation, determined by the moving correlation coefficient between MAP and the ratio of oxygenated versus deoxygenated hemoglobin (COX), was not preserved in one-third of postcardiac arrest patients [39]. Disturbed autoregulation was associated with unfavourable outcome [39, 40]. A MAP below the optimal autoregulatory range during the first 48 hours after cardiac arrest was associated with worse outcomes compared to patients with higher blood pressures [41].

The relationship between brain tissue oxygen saturation and MAP can also be used to determine the optimal MAP in individual patients after cardiac arrest. The feasibility of this technique to obtain real-time values for optimal MAP was demonstrated in a small prospective cohort study [42]. The optimal MAP for patients after cardiac arrest in this study was found to be 75 mmHg. In a retrospective study, Ameloot estimated the optimal MAP in patients after cardiac arrest to be 85 mmHg in patients with preserved autoregulation and 100 mmHg in patients with disturbed autoregulation [39].

Taken together, these results emphasize the importance of accurate blood pressure control in patients after cardiac arrest. Larger prospective cohort studies are required to establish the value of a tailored blood pressure targeted therapy versus conventional blood pressure targets.

The CBF changes after cardiac arrest. The critical closing pressure (CrCP) is a reliable method to quantify characteristics of the cerebrovascular bed and is defined as the lower limit of arterial blood pressure below which vessels collapse and flow ceases [43, 44]. Immediately following cardiac arrest, CrCP was shown to be high, accompanied by increased cerebrovascular resistance [26]. The CrCP decreased in the first 48 hours after admission towards normal values [26]. The CrCP was significantly higher in patients who survived compared to those who deceased [26]. Apparently, vasoactive tone was lost in patients with unfavourable outcome, resulting in reduced cerebrovascular resistance and a subsequent-increased CBF. In contrast, vasoactive tone and cerebral blood flow velocities returned to normal values in patients with favourable neurological outcome.

In addition, immediately following cardiac arrest, spontaneous variability of MFV was found to be low [15]. MFV variability increased to normal values in patients who survived, whereas it further decreased in patients who did not survive after cardiac arrest [15]. It is plausible that these changes are the consequence of the associated severe brain damage, resulting in impaired control of intrinsic myogenic vascular function and autonomic dysregulation. These changes in spontaneous fluctuations in MFV imply changes in dynamic cerebral autoregulation after ROSC.

Bisschops et al. showed a preserved cerebrovascular reactivity to fluctuations in $PaCO_2$ during mild therapeutic hypothermia after cardiac arrest [2]. Previously, Yenari et al. demonstrated a preserved cerebrovascular reactivity to changes in $PaCO_2$ under normothermic conditions in patients after ROSC [45]. This emphasizes the importance of strict control of blood gas values during mechanical ventilation in cardiac arrest patients, because secondary neurological damage as a result of cerebral ischaemia could be prevented by avoiding iatrogenic hypocapnia.

4. Conclusion

CBF is low after cardiac arrest and returns towards normal values in patients that ultimately survive. In patients with severe postanoxic encephalopathy disturbed autoregulation, loss of normal vascular tone, and increased CBF may contribute to the development of secondary brain damage, ultimately leading to fatal brain injury.

The changes in CBF after cardiac arrest may be regarded merely as a feature of severe primary brain damage resulting from ischaemia and reperfusion injury. Alternatively, they may contribute to the development of secondary brain damage. Whether modulation of the CBF after ROSC, for example, by maintaining MAP at optimal autoregulation ranges, impacts the outcome of these patients remains to be determined.

In addition, differences between CBF in the microcirculation are poorly understood and deserve more attention.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Time Course of Cerebrovascular Reactivity in Patients Treated for Unruptured Intracranial Aneurysms: A One-Year Transcranial Doppler and Acetazolamide Follow-Up Study

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Background. Cerebrovascular reactivity (CVR) is often impaired in the early phase after aneurysmal subarachnoid hemorrhage. There is, however, little knowledge about the time course of CVR in patients treated for unruptured intracranial aneurysms (UIA). *Methods*. CVR, assessed by transcranial Doppler and acetazolamide test, was examined within the first postoperative week after treatment for UIA and reexamined one year later. *Results*. Of 37 patients initially assessed, 34 were reexamined after one year. Bilaterally, baseline and acetazolamide-induced blood flow velocities were higher in the postoperative week compared with one year later ($p < 0.001$). CVR on the ipsilateral side of treatment was lower in the initial examination compared with follow-up (58.9% versus 66.1%, $p = 0.04$). There was no difference in CVR over time on the contralateral side (63.4% versus 65.0%, $p = 0.65$). When mean values of right and left sides were considered there was no difference in CVR between exams. Larger aneurysm size was associated with increased change in CVR ($p = 0.04$), and treatment with clipping was associated with 13.8%-point increased change in CVR compared with coiling ($p = 0.03$). *Conclusion*. Patients with UIA may have a temporary reduction in CVR on the ipsilateral side after aneurysm treatment. The change in CVR appears more pronounced for larger-sized aneurysms and in patients treated with clipping. We recommend that ipsilateral and contralateral CVR should be assessed separately, as mean values can conceal side-differences.

1. Introduction

Constriction and dilation of cerebral arterioles regulate cerebral blood flow. Cerebrovascular reactivity (CVR) reflects this regulating capacity and is a marker of cerebrovascular integrity. Impaired CVR is associated with increased risk of cerebro- and cardiovascular disease and death [1]. The temporal development of CVR has been studied in healthy subjects and in patients with cerebrovascular disease. In healthy persons, CVR is stable over time [2]. In the early phase after aneurysmal subarachnoid hemorrhage (aSAH), CVR is often impaired [3–8], especially in patients with massive hemorrhage, poor neurological status at admission, and vasospasm [9–15]. It has been suggested that transient reduction of CVR after aSAH may be associated with development of delayed cerebral ischemia and poor outcome [9, 16, 17]. There is, however, little knowledge regarding the time course of CVR in patients with unruptured intracranial aneurysms (UIA). It is unknown whether, and how, aneurysm treatment affects CVR. Information on the time course of CVR in patients treated for UIA may help in differentiating between potential effects of aneurysm treatment and the impact of an aneurysm bleeding.

The main objective of this study was to evaluate the time course of CVR in patients treated for an UIA by comparing

CVR within the first week after aneurysm treatment with CVR one year later. We further wanted to assess whether other factors like age, sex, smoking, hypertension, body mass index, aneurysm size, treatment side, or treatment modality were associated with the stability of CVR over time.

2. Methods

2.1. Participants and Time Scheme. In a previous study, we analyzed early postoperative CVR data from patients treated for UIA in the Department of Neurosurgery, Haukeland University Hospital, between February 2011 and May 2013 [18]. The patients were treated with either endovascular coiling or surgical clipping, and they were examined within the first week after aneurysm treatment. In the present study, CVR was reevaluated in the same patients one year after aneurysm treatment. Exclusion criteria were identical to those used in the previous study: former treatment of intracranial aneurysms; nonsaccular aneurysms; giant aneurysms treated with proximal artery occlusion; carotid stenosis (>50%) or occlusion; lack of transtemporal bone window in transcranial Doppler examination; and contraindications to acetazolamide (e.g., sulfonamide allergy, adrenal or pituitary insufficiency, and kidney or liver failure).

Demographics, aneurysm location, and treatment were recorded, as well as body mass index, smoking status, and hypertension (previously diagnosed and treated or systolic pressure > 140 mmHg and/or diastolic pressure > 90 mmHg persistently observed during admission). Aneurysm size was measured using the following parameters: maximum diameter of the dome, independent of angles and directions (maximum diameter, Dmax), maximum diameter of the dome, perpendicular to the aneurysm height (width, W), maximum height from dome tip perpendicular to aneurysm neck (height, H), and diameter of the aneurysm neck (neck, N). Aspect ratio (H/N) and bottleneck ratio (W/N) were calculated [19, 20].

The study was conducted in accordance with the Declaration of Helsinki (2013) of the World Medical Association and was approved by the local ethics committee. All patients gave written informed consent.

2.2. Cerebrovascular Reactivity. CVR testing was performed using transcranial Doppler (TCD) monitoring of blood flow velocities in the middle cerebral arteries (MCA) before, during, and after intravenous injection of acetazolamide (AZ). The method has previously been described in detail [18]. Except for an additional manufacturer of AZ, the method of CVR testing was identical to the initial study [18]. The AZ manufacturers used in this study were Goldshield Ltd., Croydon, Surrey, UK; Sanofi Aventis, Paris, France; and Mercury Pharmaceuticals Ltd., Croydon, Surrey, UK. The AZ dose was 1000 mg for patients weighing < 80 kg, and 15 mg/kg for patients weighing ≥ 80 kg. The maximum dose was 1500 mg. All examinations in the initial and follow-up study were performed by the same sonographer (MLB).

Cerebrovascular reactivity was defined as the maximum percentage change in mean blood flow velocity (MFV) after administration of AZ: $\text{CVR}\,(\%) = [(\text{MFV}_{\text{AZ}} - \text{MFV}_{\text{BASELINE}})/\text{MFV}_{\text{BASELINE}}] \times 100$, where CVR is cerebrovascular reactivity, $\text{MFV}_{\text{BASELINE}}$ is baseline mean blood flow velocity (before AZ), and MFV_{AZ} is maximum mean blood flow velocity after AZ.

2.3. Statistical Analysis. Two measures of central tendency and dispersion were used: mean and standard deviation (SD) for variables that were symmetric around the mean, and median and interquartile range (IQR) for those that were nonsymmetric. In cases where patients underwent treatment for multiple aneurysms during the same procedure, averaged aneurysm size was used in the analyses. The relationships between blood flow velocities and CVR at the time of initial examination and one year later were studied using paired t-tests. Paired t-test was also used to assess possible differences in velocities and CVR related to side (right/left and ipsilateral/contralateral to the aneurysm treatment). Regarding treatment modality (clipping or coiling), two-sample t-tests were used. To simplify analyses, patients with midline aneurysms were allocated to the side chosen for endovascular or surgical approach. As in the previous study [18], mean CVR of the two sides (right and left) was calculated for all individuals. If the measurement on one side was missing, the mean CVR was set to the nonmissing value. Simple linear regressions, stratified on treatment modality, were conducted on mean CVR at follow-up versus mean CVR at first examination. Also, difference in mean CVR between first exam and follow-up was the outcome in a multiple regression and a set of simple linear regressions. Covariates were age, sex, hypertension, smoking, body mass index, weight difference from initial exam to follow-up, treatment modality, maximum aneurysm diameter (Dmax), mean CVR at the time of initial examination, and difference in mean AZ dose per kg from initial exam to follow-up. Lastly, maximum aneurysm diameter was included as covariate in a simple linear and multiple regression with CVR at the time of the initial examination as outcome, in addition to the covariates tested in a previous report (age, sex, hypertension, smoking, body mass index, and treatment modality) [18]. The regression analyses were repeated with ipsilateral CVR as outcome variable instead of mean CVR, and with stratification for age, sex, and treatment modality.

All statistical analyses were performed with R version 3.4.3 [21].

3. Results

3.1. Patients, Aneurysm, and Treatment. Of 37 patients examined in the initial study, two patients chose to abstain from the follow-up test due to side effects of AZ at the initial examination, and one patient did not meet for follow-up due to long travel to the hospital. This left us with a study population of 34 patients.

Table 1 shows patient characteristics. Weight difference is the difference in body weight from the first examination to follow-up. All other variables listed in the table were recorded at the time of aneurysm treatment. Mean age was 49.0 (SD 9.6, range 27–65) years. In 20 of the 34 patients (58.8%) the body

Table 1: Patients characteristics.

	(n = 34)
Age, years[a]	49.0 (9.6)
Height, cm[a]	169.2 (8.6)
Weight, kg[a]	76.1 (15.5)
BMI, kg/m^2[a]	26.5 (4.8)
Weight difference, kg[a]	0.4 (4.7)
Female[b]	22 (64.7)
Hypertension[b]	15 (44.1)
Smoking[b]	
Current	19 (55.9)
Previous	11 (32.4)
Never	4 (11.8)

[a]mean (SD); [b]n (%); BMI: body mass index; SD: standard deviation.

weight was different at the time of follow-up compared with the first examination (range 10 kg reduction–13 kg increase). In 11 patients (32.4%) the weight difference was >2 kg. Table 2 shows aneurysm and treatment characteristics.

3.2. Cerebrovascular Reactivity. In total, 56 bilateral and 12 unilateral examinations were performed in the 34 patients. Unilateral examinations were more common in the initial exams (23.5%) compared with follow-up (11.8%), presumably because postoperative intracranial air can cause insufficient insonation. Median time between treatment and initial exam was 51.0 (IQR 39.5) hours. Median time between treatment and follow-up exam was 376.5 (IQR 31.8) days. Median time between initial examination and follow-up was 374.5 (IQR 29.3) days.

Of 68 examinations, 42 (61.8%) were performed using 1000 mg AZ. The remaining 26 examinations (38.2%) were done with 15 mg AZ per kg because of high bodyweight. Mean bodyweight was 76.1 (SD 15.5, range 40 to 110) kg at the time of the first examination and 76.6 (SD 17.2, range 40 to 118) kg at follow-up. Mean AZ dose was 15.1 (SD 2.3) mg/kg in the first examination and 15.1 (SD 2.4) mg/kg at follow-up. There was no correlation between AZ dose per kg and CVR (Pearson's $R = 0.08$, $p = 0.66$ in the first examination, and $R = -0.11$, $p = 0.52$ at follow-up).

Table 3 shows blood flow velocities and CVR results. In the initial examination MFV in the middle cerebral arteries was 58.6 cm/s before stimulation with AZ and 94.3 cm/s after, giving a mean CVR of 62.7%. Follow-up testing showed MFV 51.4 cm/s before AZ, 84.4 cm/s after, and mean CVR of 65.6%. Bilaterally, baseline and AZ-induced blood flow velocities were higher in the postoperative week compared with 1 year after aneurysm treatment ($p \leq 0.009$ in all situations). When assessing mean values of the right and left sides, no difference between CVR at first examination and follow-up was found ($p = 0.31$). When assessing CVR according to treatment laterality, there was no difference over time on the contralateral side (65.0% at follow-up versus 63.4% at the initial examination, $p = 0.65$). However, on the ipsilateral side of aneurysm treatment there was an apparent change in CVR over time. Ipsilateral CVR was 58.9% (SD 19.3) in the initial examination versus 66.1% (SD 18.5) at follow-up ($p = 0.04$), corresponding to an absolute increase of 7.2% and relative increase of 12%. Subgroup analyses for treatment modalities had lower sample sizes, and the significance disappeared ($p = 0.16$). CVR change on the ipsilateral side seemed larger in patients treated with clipping compared with patients treated with coiling (absolute increase 10.5 versus 4.7%, and relative increase 17 versus 8%), yet the number of patients in each subgroup is low ($n = 12$ for clipping and $n = 22$ for coiling) and results are inconclusive ($p = 0.42$). The same trend was found for mean CVR values of the right and left side, with an absolute and relative increase of 8.7% and 13% in patients treated with clipping versus 0.2% absolute reduction and 0% relative change in patients treated with coiling ($p = 0.18$). The tendency of larger CVR difference between exams was present for all patients treated with aneurysm clipping, regardless of whether temporal clipping of a parent artery was performed or not. However, despite similar values for CVR difference between exams, patients treated with temporal clipping appeared to have higher CVR values compared with patients treated with "standard" clipping (without the need for temporary clipping), at both the initial exam and follow-up. Due to few observations, statistical power is however insufficient to evaluate potential differences within the clipping subgroup.

Table 4 shows the results of the regression analyses regarding the relationship between difference in mean CVR from the first examination to follow-up and several different variables. In the simple analysis, maximum aneurysm diameter and CVR in the first examination were associated with a change in CVR. An 1 mm increase in the maximum diameter of the aneurysm dome was associated with an increase in CVR difference by 3.2 and 2.5 percentage points in the simple and multiple regressions, respectively ($p = 0.005$ and $p = 0.04$). For every percentage point increase in CVR in the first examination, the change in CVR from initial exam to follow-up was reduced with 0.3 percentage points ($p = 0.05$). The association was stronger in the multiple model, where the reduction in CVR change was 0.5 percentage points for every percentage point increase in CVR in the first examination ($p = 0.01$). In the multiple analyses age and treatment modality were also associated with change in CVR. For age, the change in CVR increased with 0.8 percentage points per year ($p = 0.03$). For treatment modality, the multiple model showed that patients treated with clipping had 13.8 percentage points increased change in CVR compared with patients treated with coiling ($p = 0.03$). There were no associations between change in CVR and sex, body mass index, body weight difference between exams, hypertension, and smoking. There were no major changes in the results when regression analyses were repeated after stratification for sex, age (≤50 years versus >50 years), and treatment modality (coiling versus clipping).

Regression analyses were also repeated with ipsilateral CVR as outcome variable instead of mean CVR. Patients with missing CVR values on the ipsilateral side, at the time of either the first exam or follow-up, were excluded. This applied to 7 of 34 (20.6%) patients: 2 of 22 (9.1%) patients treated with coiling and 5 of 12 (41.7%) patients treated with clipping.

Table 2: Aneurysm and treatment characteristics.

	$n = 34$
Multiple aneurysms, *n* (%)	12 (35.3)
Treatment modality, *n* (%)	
Coil	22 (64.7)
Clip[a]	12 (35.3)
Treatment side, *n* (%)[b]	
Left	18 (52.9)
Right	16 (47.1)
Location of treated aneurysms, *n* (%)	
MCA	14 (41.2)
ICA, incl. ophthalmic artery and PCOM	10 (29.4)
ACOM, anterior complex, and pericallosal artery	7 (20.6)
Basilar top, cerebelli superior, PICA, VB, and distal posterior	3 (8.8)
Size of treated aneurysms, mean (SD)[c]	
Maximum diameter (*D*max), mm	6.3 (2.4)
Height (*H*), mm	6.4 (2.8)
Neck (*N*), mm	4.1 (1.8)
Width (*W*), mm	5.4 (2.2)
Aspect ratio (*H*/*N*)	1.6 (0.5)
Bottleneck ratio (*W*/*N*)	1.4 (0.5)

[a]In four of twelve patients temporal clipping of a parent artery was performed; [b]one patient treated with combined clipping of an ACOM aneurysm and a right MCA aneurysm in one procedure was allocated to the right side. Eight patients with midline aneurysms (ACOM and basilar top) were allocated to the chosen side of approach; [c]the majority of patients received treatment for a single aneurysm. For the 4 of 34 patients (11.8%) that underwent treatment for two aneurysms during the same procedure, aneurysm size was averaged; ACOM: anterior communicating artery; ICA: internal carotid artery; MCA: middle cerebral artery; PCOM: posterior communicating artery; PICA: posterior inferior cerebellar artery; VB: vertebrobasilar artery; maximum diameter (*D*max): maximum diameter of the dome (independent of angles and directions); height (*H*): maximum height from dome tip perpendicular to aneurysm neck; neck (*N*): diameter of the aneurysm neck; width (*W*): maximum diameter of the dome, perpendicular to the aneurysm height (*H*).

The lower sample size in the analyses with ipsilateral CVR as outcome yielded more uncertainty. Apart from higher p values, findings were primarily consistent with the results of the original regressions using mean CVR as outcome. Maximum aneurysm diameter and CVR at first exam were still associated with change in ipsilateral CVR between exams in the simple analysis ($p = 0.05$), whereas the multiple analysis provided weaker evidence for such associations ($p = 0.22$ for aneurysm diameter and $p = 0.10$ for first CVR). The positive association between change in CVR and age and clipping found in the regression with mean CVR was less obvious in the regression with ipsilateral CVR. The estimate for age was 0.7 ($p = 0.10$) versus 0.8 ($p = 0.03$) in the regression with mean values. The estimate for clipping was 9.8 ($p = 0.19$) in the regression with ipsilateral values versus 13.8 ($p = 0.03$) in the regression with mean values.

Finally, regression analyses were performed to assess if larger aneurysm diameter was correlated with lower initial CVR. When only results from the initial exam were included in the statistical analyses, fewer observations yielded high p values. Maximum aneurysm diameter had an estimate of −0.6 in the simple analysis ($p = 0.61$) and −1.7 in the multiple analysis ($p = 0.15$). Few observations hamper the assessment of a possible association between larger aneurysm size and reduced CVR in the first week after aneurysm treatment.

Figure 1 shows a scatter plot of mean CVR at first exam and follow-up. The regression lines for the two treatment modalities are almost parallel, with the line for patients treated with clipping shifting up about 8 to 15 percentage points. This is in accordance with Table 4.

Figure 2 shows box plots comparing change in mean CVR from first exam to follow-up in patients treated with coiling and clipping. Although there was little evidence for difference in mean CVR change in patients treated with coiling compared with clipping ($p = 0.14$), Figure 2 hints that patients treated with clipping had a greater difference between the initial examination and follow-up. Still, the evidence is inconclusive.

Diamox Goldshield Ltd. was used in 44 CVR tests (64.7%), Sanofi Aventis in 20 tests (29.4%), and Mercury Pharmaceuticals Ltd. in 4 tests (5.9%). There did not seem to be any important differences in CVR between the three manufacturers (Mercury versus Goldshield: $p = 0.07$; Mercury versus Sanofi Aventis: $p = 0.84$; Goldshield versus Sanofi Aventis: $p = 0.46$).

4. Discussion

4.1. Main Findings. In this study, we found a lower CVR on the ipsilateral side of aneurysm treatment in the post-operative week compared with one-year follow-up. There was no evidence of any difference in CVR over time when mean values of the right and left sides were assessed. Larger aneurysm size is associated with increased change in CVR.

Table 3: Blood flow velocities and cerebrovascular reactivity at baseline and follow-up.

	First exam mean (SD)	Follow-up mean (SD)	Absolute difference	Relative difference	p
$MFV_{BASELINE}$ (cm/s)					
Ipsilateral	59.8 (13.8)	51.2 (13.3)	−8.6	0.86	<0.001
Contralateral	57.7 (14.7)	52.0 (12.1)	−5.7	0.90	0.003
Mean	58.6 (13.2)	51.4 (11.7)	−7.2	0.88	<0.001
MFV_{AZ} (cm/s)					
Ipsilateral	93.6 (18.0)	84.4 (20.2)	−9.2	0.90	0.001
Contralateral	93.4 (23.3)	85.0 (20.1)	−8.4	0.91	0.009
Mean	94.3 (20.0)	84.4 (18.5)	−9.9	0.90	<0.001
ΔMFV (cm/s)					
Ipsilateral	33.8 (9.2)	33.1 (9.8)	−0.7	0.98	0.43
Contralateral	35.7 (11.2)	33.0 (11.8)	−2.7	0.93	0.15
Mean	35.7 (10.2)	33.1 (9.9)	−2.7	0.93	0.09
CVR (%)					
Ipsilateral	58.9 (19.3)	66.1 (18.5)	7.2	1.12	0.04
Contralateral	63.4 (17.5)	65.0 (23.6)	1.7	1.03	0.65
Mean	62.7 (17.2)	65.6 (19.4)	2.9	1.05	0.31
MV (n)					
Ipsilateral	7	3			
Contralateral	1	1			

CVR: cerebrovascular reactivity; $MFV_{BASELINE}$: baseline mean blood flow velocity (before acetazolamide); MFV_{AZ}: maximum mean blood flow velocity after acetazolamide; ΔMFV: absolute change in mean flow velocity after acetazolamide; MV: missing value; p: p value from paired t-test (follow-up–first); SD: standard deviation. Note that if one side had a missing value, the mean is just the remaining value. This is why the mean is not simply the mean of ipsilateral and contralateral values. Patients with MV were excluded from the paired t-test. The sample size was therefore reduced in the analyses of ipsilateral and contralateral values (ipsilateral $n = 27$, contralateral $n = 33$, mean $n = 34$).

Table 4: Regression results: difference in mean CVR between exams versus a number of variables.

	Simple		Multiple	
	Estimate	p	Estimate	p
Age, years	0.1	0.68	0.8	0.03
Female	−4.9	0.42	−4.5	0.46
BMI, kg/m^2	−0.2	0.78	−0.1	0.84
Weight difference, kg	0.3	0.63	0.3	0.81
Hypertension	1.7	0.78	3.3	0.58
Smoking				
Current	ref	-	ref	-
Previous	−2.8	0.67	−2.8	0.62
Never	−5.2	0.59	−0.7	0.94
Treatment modality				
Coil	ref	-	ref	-
Clip	9.0	0.14	13.8	0.03
Maximum aneurysm diameter (Dmax)	3.2	0.005	2.5	0.04
Mean CVR at first exam, %	−0.3	0.04	−0.5	0.01
Difference in AZ dose per kg	−2.4	0.49	−1.9	0.81

AZ: acetazolamide; BMI: body mass index; CVR: cerebrovascular reactivity; Dmax: maximum diameter of the aneurysm dome (independent of angles and directions); ref: reference.

In addition, results suggest that the difference in CVR may be greater in patients treated with clipping compared with coiling, but evidence is inconclusive.

In a previous study, where CVR was examined in the first week after aneurysm treatment, we did not find any difference when comparing treated and untreated sides (59.4% versus 63.0%, $p = 0.16$) [18]. We concluded that CVR in patients with UIA did not differ from normal values reported in healthy subjects and that findings did not indicate a systemically impaired vascular system in patients with UIA. New

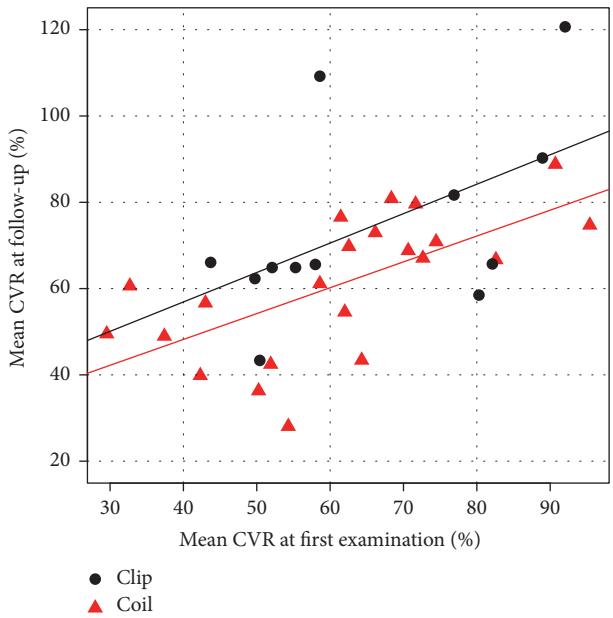

Figure 1: Scatter plot of mean cerebrovascular reactivity (CVR) at the time of initial examination and follow-up, together with regression lines. Results for patients treated with coiling are marked with red triangles, and results for patients treated with clipping are marked with black dots.

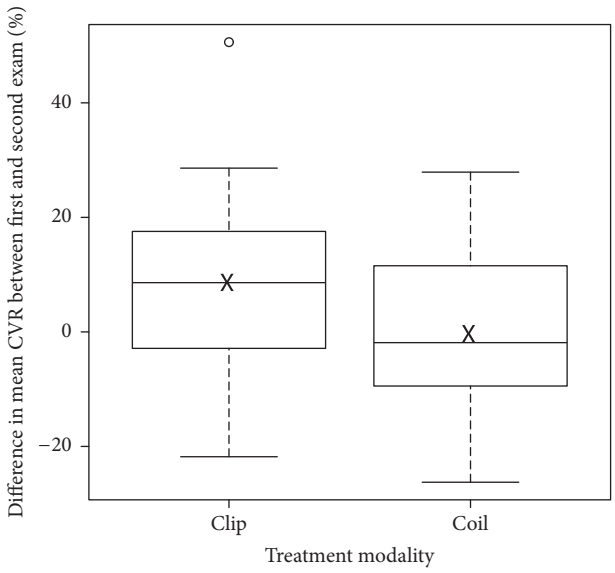

Figure 2: Box plots comparing change in mean cerebrovascular reactivity (CVR) from the first examination to follow-up in patients treated with coiling and clipping. Boxes extend from the 25th to the 75th percentile. Horizontal bars represent the median, and whiskers extend to the most extreme point that is less than 1.5 times the interquartile range from the box. Mean values are marked with crosses, and a single outlier is depicted as a circle.

information based on results from follow-up testing one year later now indicates that there may be a side difference in CVR after aneurysm treatment after all. Ipsilateral CVR was 58.9% after treatment versus 66.1% one year later, and contralateral CVR was 63.4% after treatment versus 65.0% one year later. The postoperative CVR of 58.9% seems to stand out as lower than the other CVR values, indicating a temporary reduction in CVR on the treated side. Even though we could not rule out the fact that the trend with lower CVR on the ipsilateral side was due to chance when only postoperative results were assessed ($p = 0.16$) the difference was more pronounced when follow-up results were included (difference in ipsilateral CVR over time, $p = 0.04$). Still, the sample size is limited and results must be interpreted with caution.

Furthermore, this study showed higher baseline and AZ-induced blood flow velocities in the postoperative week compared with one year after aneurysm treatment. The increased velocities can be explained by postoperative hyperemia. Transient hyperemia is common after craniotomy [22]. However, hyperemia has previously only been found in the first postoperative hour [22], and the median time for the first CVR testing in our study was 51.0 (IQR 39.5) hours after treatment. To our knowledge, there have been no reports about transient hyperemia after endovascular aneurysm treatment. Alternatively, posttreatment spasm could be the cause of elevated blood flow velocities at the initial exam. Moreover, comparison of absolute blood flow velocities is problematic as the probe positioning and insonation angle probably were different at the time of the initial and follow-up exam. Still, there is no reason why altered insonation angle and probe positioning should only cause increased velocities. In theory, changes in technical insonation aspects could just as well cause a reduction of measured velocities.

Our finding of reduced CVR on the ipsilateral side after aneurysm treatment may be related to postoperative hyperemia. It is possible that a transient hyperemia in response to aneurysm clipping or coiling affects the arteriolar vasodilating capacity and thus influences CVR. Baseline velocities ($MFV_{BASELINE}$) were higher at first exam compared with one-year follow-up (59.8 cm/s versus 51.2 cm/s, $p < 0.001$), whereas the absolute change in velocity (ΔMFV) was the same at the two examination times (33.8 cm/s versus 33.1 cm/s, $p = 0.43$). Since CVR is defined as percentage change in velocity after AZ compared with baseline, the same ΔMFV yields lower CVR values when baseline velocities are increased (unchanged numerator and higher denominator of the fraction). An alternative explanation for decreased CVR after aneurysm treatment may be that harboring an aneurysm in itself impairs CVR. This effect may still be present in the first postoperative days, while vasodilating capacity can be restored after aneurysm treatment and CVR normalized one-year later. Since TCD and AZ testing were not performed prior to aneurysm treatment it is difficult to say if the reduction in CVR is caused by the aneurysm itself or by aneurysm treatment.

4.2. Time Course of CVR in Healthy Subjects. Schwertfeger et al. (2006) assessed the time course of CVR in healthy subjects [2]. TCD and AZ were used to investigate CVR in 33 healthy subjects at baseline and after 1 to 3 years (mean 21.6 months). They performed unilateral testing and found no changes in CVR over time. Like in our study, they did not find any association between sex and smoking and CVR

change. Unlike their findings, we found a positive association between age and change in CVR from first examination to follow-up ($p = 0.03$ in the multiple regression model). The possible influence by age on CVR is unclear, and studies in healthy subjects have shown varying effects [23–30].

4.3. Time Course of CVR in Patients with Intracranial Aneurysms. Several studies have assessed the time course of CVR in patients with ruptured intracranial aneurysms. CVR is often impaired in the early phase after aSAH [3–6], especially in patients with massive hemorrhage, poor neurological status at admission, and vasospasm [9–15]. There is a possible association between progressive impairment in CVR in the early phase after aSAH and subsequent development of delayed cerebral ischemia [9, 16]. Transient reduction of CVR may also be associated with poor outcome [17]. Follow-up studies months and years after aSAH have shown normalization of CVR, regardless of the severity of hemorrhage and presence of vasospasm in the acute phase [31–33]. In contrast to the numerous studies on CVR in patients with aSAH there are few studies addressing CVR in patients with UIA. To our knowledge only one study has used AZ test and TCD to assess CVR in this patient group [18], and six reports have used CO_2 as vasoactive stimuli [3, 4, 7, 14, 15, 33]. In these studies, CVR testing was performed either at a single time-point after aneurysm treatment [18, 33] or at multiple time-points within 24 hours in close relation to the time of aneurysm surgery [3, 4, 7, 14, 15]. To our knowledge, this is the first study to investigate within-subject differences over time in patients treated for UIA. We found evidence in favor of a transient reduction of CVR on the ipsilateral side of aneurysm treatment, and recommend separate assessment of ipsilateral and contralateral CVR as mean values of right and left sides can conceal side-differences. Our finding of possible transient reduction of CVR also after treatment for *unruptured* aneurysms provides valuable insight and may enable better interpretation of CVR results after aneurysm treatment.

Studies with measurement of CVR at a single time-point have not shown any association between CVR and aneurysm treatment modality [18, 31], whereas in this follow-up study a possible association between treatment modality and change in CVR was found. Patients treated with aneurysm clipping appeared to have a larger difference in CVR between exams. This tendency was present for all patients treated with aneurysm clipping, regardless of whether temporal clipping of a parent artery was performed or not. Still, the number of patients in the subgroups for treatment modality is low. In particular, the number of observations in patients treated with clipping is reduced due to missing values, presumably related to postoperatively intracranial air. Results should thus be interpreted with caution.

4.4. Technical Considerations. Blood flow velocities demonstrate diurnal variations [34]. The follow-up examination was not performed at the identical time of day as the initial CVR test, but the time differences between exams were small (median 2.25 hours, IQR 2.56 hours) and we consider their influence as negligible. Mean AZ dose was the same in the first examination and follow-up (mean 15.1 mg/kg, SD 2.3 and 2.4 mg/kg, respectively). A third of the patients had a weight difference of >2 kg from first exam to follow-up. Nonetheless, the weight difference was small (mean 0.4 kg, SD 4.7 kg) and there was no association between weight difference and change in CVR ($p = 0.93$). We used different brands of AZ, but there was no differences in CVR between the three manufacturers. As expected, insufficient insonation due to postoperative intracranial air was more common on the ipsilateral side of aneurysm clipping compared with the contralateral side, or compared with patients treated with coiling.

4.5. Strengths and Limitations. To our knowledge, this is the first study to investigate within-subject differences in CVR over time in patients treated for UIA. The sample size is in the upper range compared with CVR studies in healthy subjects [2, 35–46]. The method of testing was identical at initial examination and follow-up. We used the same sonographer (MLB) in all examinations to reduce operator variability, as the intrarater reproducibility for TCD examinations has been found superior to interrater [47, 48].

The AZ dose should ideally have been bodyweight-based in all patients in the study, not only in patients weighing ≥80 kg. Still, the recommended AZ dose of 13 to 18 mg/kg [39, 49] was achieved for the vast majority of patients (91.2%). Blood flow velocities are affected by physiological factors such as hematocrit, arterial CO_2 tension, heart rate, and mean arterial pressure [50], and hyperventilation can theoretically counteract the vasodilatory effect of AZ. We did not routinely monitor these parameters in our study.

Even though the sample size is rather large compared with other CVR studies, the limited number of patients makes it difficult to draw definite conclusions regarding regression results and subgroup effects, especially for treatment modality. The regression analyses based on ipsilateral CVR were hampered by missing values in several patients, especially in patients treated with clipping. Subgroup analysis based on laterality and treatment modality should be considered when planning the sample size of future studies.

To best assess the effect of aneurysm treatment on CVR it would have been preferable to examine patients before and after the procedure. Patients were not examined before aneurysm treatment in our study, partly because this study was part of a larger study where the set-up was designed for comparison of CVR in patients treated for ruptured and unruptured aneurysms, and partly because we wanted to avoid test-induced aneurysm rupture, a highly unlikely yet serious complication.

5. Conclusions

This study implies that patients with UIA may have a temporary reduction in CVR on the ipsilateral side after aneurysm treatment. The change in CVR is associated with larger aneurysm size and is possibly more pronounced in patients treated with clipping. We recommend that results from ipsilateral and contralateral sides should be assessed separately as mean values can conceal side-differences in CVR.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Pinocembrin Protects Blood-Brain Barrier Function and Expands the Therapeutic Time Window for Tissue-Type Plasminogen Activator Treatment in a Rat Thromboembolic Stroke Model

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Tissue-type plasminogen activator (t-PA) remains the only approved therapy for acute ischemic stroke but has a restrictive treatment time window of 4.5 hr. Prolonged ischemia causes blood-brain barrier (BBB) damage and increases the incidence of hemorrhagic transformation (HT) secondary to reperfusion. In this study, we sought to determine the effect of pinocembrin (PCB; a pleiotropic neuroprotective agent) on t-PA administration-induced BBB damage in a novel rat thromboembolic stroke model. By assessing the leakage of Evans blue into the ischemic hemisphere, we demonstrated that PCB pretreatment 5 min before t-PA administration significantly reduced BBB damage following 2 hr, 4 hr, 6 hr, and even 8 hr ischemia. Consistently, PCB pretreatment significantly decreased t-PA infusion-resulting brain edema and infarction volume and improved the behavioral outcomes following 6 hr ischemia. Mechanistically, PCB pretreatment inhibited the activation of MMP-2 and MMP-9 and degradation of tight junction proteins (TJPs) occludin and claudin-5 in the ischemic hemisphere. Moreover, PCB pretreatment significantly reduced phosphorylation of platelet-derived growth factor receptor α (PDGFRα) as compared with t-PA alone. In an *in vitro* BBB model, PCB decreased transendothelial permeability upon hypoxia/aglycemia through inhibiting PDGF-CC secretion. In conclusion, we demonstrated that PCB pretreatment shortly before t-PA infusion significantly protects BBB function and improves neurological outcomes following prolonged ischemia beyond the regular 4.5 hr t-PA time window. PCB pretreatment may represent a novel means of increasing the safety and the therapeutic time window of t-PA following ischemic stroke.

1. Introduction

Thrombolysis with tissue-type plasminogen activator (t-PA) is the only FDA-approved therapy for acute ischemic stroke; however, it has a narrow therapeutic time window of 3 to 4.5 hours after cerebral ischemia onset [1]. Delayed t-PA treatment after prolonged ischemia leads to severe complications such as hemorrhagic transformation (HT), brain edema, and increased mortality [2]. Because of the narrow therapeutic window and potential severe complications, t-PA treatment is applied to less than 5% of ischemic stroke patients [3]. Although the mechanisms underlying t-PA-induced HT are still unclear, blood-brain barrier (BBB) damage can cause HT [2, 4–6]. BBB disruption after ischemic stroke is a dynamic process that is characterized by the initial damage during ischemia and a secondary injury during reperfusion

[2]. Prolonged ischemia leads to severe BBB damage that dramatically increases the risk of HT after t-PA thrombolysis. Therefore, developing a novel adjuvant therapeutic strategy to protect BBB integrity and extend therapeutic time window of t-PA during ischemia is critical for improving the outcome of stroke treatment.

Pinocembrin (5,7-dihydroxyflavanone, PCB) is a natural flavonoid compound which is found in honey, propolis, and plants including ginger roots and wild marjoram [7–9]. With the primary target remaining unknown, PCB has shown potent anti-inflammatory and neuroprotective effects through reducing reactive oxygen species (ROS) and apoptosis, modulating mitochondrial function, and protecting the BBB in various animal ischemic stroke models [8–11]. Moreover, PCB ameliorated neuroinflammation and reduced lesion volume in a collagenase-induced intracerebral hemorrhage model and traumatic brain injury model [12, 13]. However, whether PCB is protective in a clinically relevant ischemic stroke model, in which the cerebral artery is occluded by thrombus and reperfused by t-PA thrombolysis, remains unknown. In this study, we observed that PCB was rapidly distributed into the cerebrospinal fluid (5–7 min) and alleviated BBB breakdown induced by t-PA-mediated cerebral ischemia/reperfusion injury in rats. We investigated to what extent and how PCB could mitigate ischemic BBB damage and extend the therapeutic time window of t-PA in a novel rat model of thromboembolic stroke we developed previously [14, 15].

2. Materials and Methods

2.1. Animal Model of Thromboembolic Stroke. All procedures were approved by the Institutional Animal Care and Use Committee of the Peking Union Medical College and in accordance with the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley (SD) rats (250 to 300 g) were purchased from Vital River Laboratory Animal Technology Co., Beijing. The rats were anesthetized with 3% isoflurane, and the anesthesia was maintained with 1.0% to 1.5% gaseous isoflurane. Rectal temperature was maintained between 37.0°C ± 0.5°C using a feedback-controlled heating system. The MCA of male SD rats was occluded by a thrombus formed within the common carotid artery (CCA) by constant galvanic stimulation, as we previously described [14, 15]. Briefly, the common carotid artery (CCA) was dissected and the galvanic stimulation (1.00 mA) was initiated and sustained for 225 s. The thrombus was smashed 10 times with ophthalmic forceps with a serrated soft tip and flushed into the middle cerebral artery (MCA)/lacunar artery by Willis circulation.

The successful occlusion was confirmed by monitoring cerebral focal perfusion with laser Doppler. Only rats that showed sustained ischemia with less than 25% of the pre-embolic baselines were included. This embolic stroke model is directly relevant to thromboembolic ischemia in patients, which allowed us to compare the effects of pinocembrin on t-PA's complications under identical and controlled ischemia and reperfusion conditions.

2.2. Experimental Design. To investigate the effect of PCB on the progression of BBB damage and its effect on the neurovascular complications of delayed t-PA treatment, we chose four ischemia durations: 2 hours, 4 hours (within the established 3~4.5-hour thrombolytic time window), 6 hours, and 8 hours (outside the window). t-PA was intravenously infused for 30 min and the brain tissue was collected 2 hours after t-PA infusion.

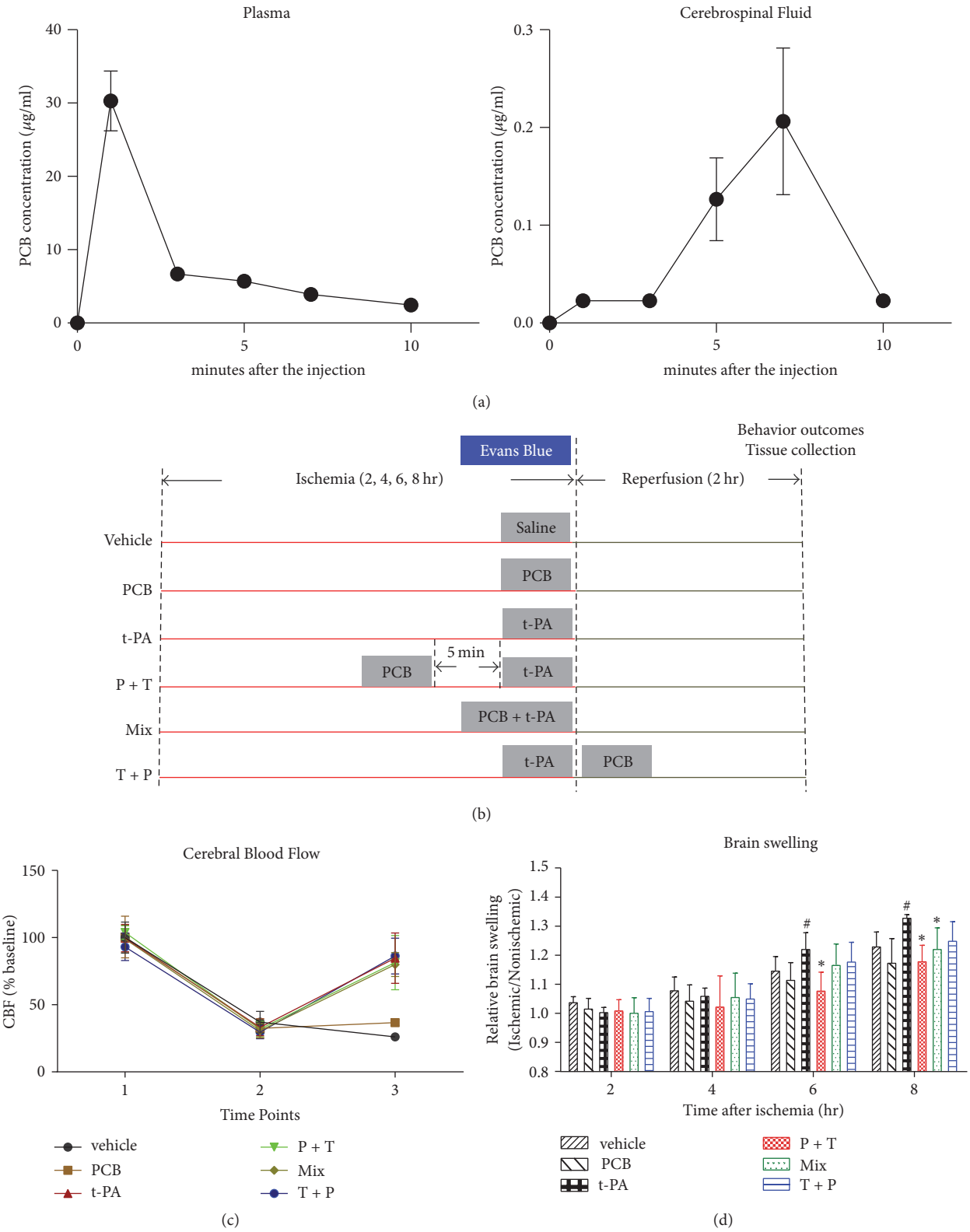
Rats with successful occlusion were randomly assigned to 6 groups: vehicle, t-PA ($n = 40$), PCB ($n = 40$), P + T ($n = 40$), T + P ($n = 40$), and mixture ($n = 40$). Each group was further divided into 4 subgroups with 2-, 4-, 6- or 8-hour ischemia followed by 2-hour reperfusion. The experimental design is schematically illustrated in Figure 1. Another set of rats were used for long-term (7 days) behavioral tests and mortality: vehicle ($n = 10$), PCB ($n = 10$), t-PA ($n = 10$), and PCB + t-PA ($n = 10$) were administered after 6 hr ischemia. After the initial administration, PCB and P + T group received PCB alone every 24 h, while vehicle or t-PA groups received saline, for another 6 days.

We chose to evaluate the impact of PCB on BBB protection at 2 hours after reperfusion for three reasons: (1) ischemia/reperfusion and t-PA-associated BBB damage occurred rapidly within the first several hours after reperfusion starts [16, 17]; (2) t-PA treatment significantly increased BBB damage as early as the 2 hr poststroke time point [17]; (3) the number of animals needed is minimized as prolonged reperfusion causes high mortality rate.

2.3. Reagents Administration. PCB was synthesized by the Department of Medicinal Chemistry of Chinese Academy of Medical Sciences (MW: 255.25; chromatographic purity > 99%). Human recombinant t-PA (alteplase, Boehringer-Ingelheim, Germany) was infused into rats through the right femoral vein at 2, 4, 6, or 8 hours after the occlusion onset, to the P + T, T + P, or mixture (Mix) groups. In the P + T group, PCB was injected 5 min before t-PA infusion. In the T + P group, PCB was injected right after the t-PA infusion, and in the Mix group PCB and t-PA were premixed before the administration. The vehicle used in this study was stroke-physiological saline solution (SPSS). PCB was used as 10 mg/kg *in vivo* and 1 μM *in vitro*. t-PA was used as 1 mg/kg *in vivo* and 10 μg/ml *in vitro*. All the experiments were performed in a blinded manner. The behavior tests and agents' administration were performed by different members of the group to avoid unintentional bias.

2.4. Cerebral Distribution of PCB. PCB was injected through the caudal vein in rat. The blood and cerebral spinal fluid were collected at 1, 3, 5, 7, and 10 min after the injection. The analyses were performed on an Agilent Zorbax SB-C18 chromatographic column (5 μm, 4.6 × 250 mm) at 35°C with an Agilent Zorbax SB-C18 precolumn. The chromatographic conditions and sample preparation were in accordance with a previous report [18].

2.5. Measurement of Behavior Outcomes. At the end of the 2-hour reperfusion, the neurological deficits test, rotating



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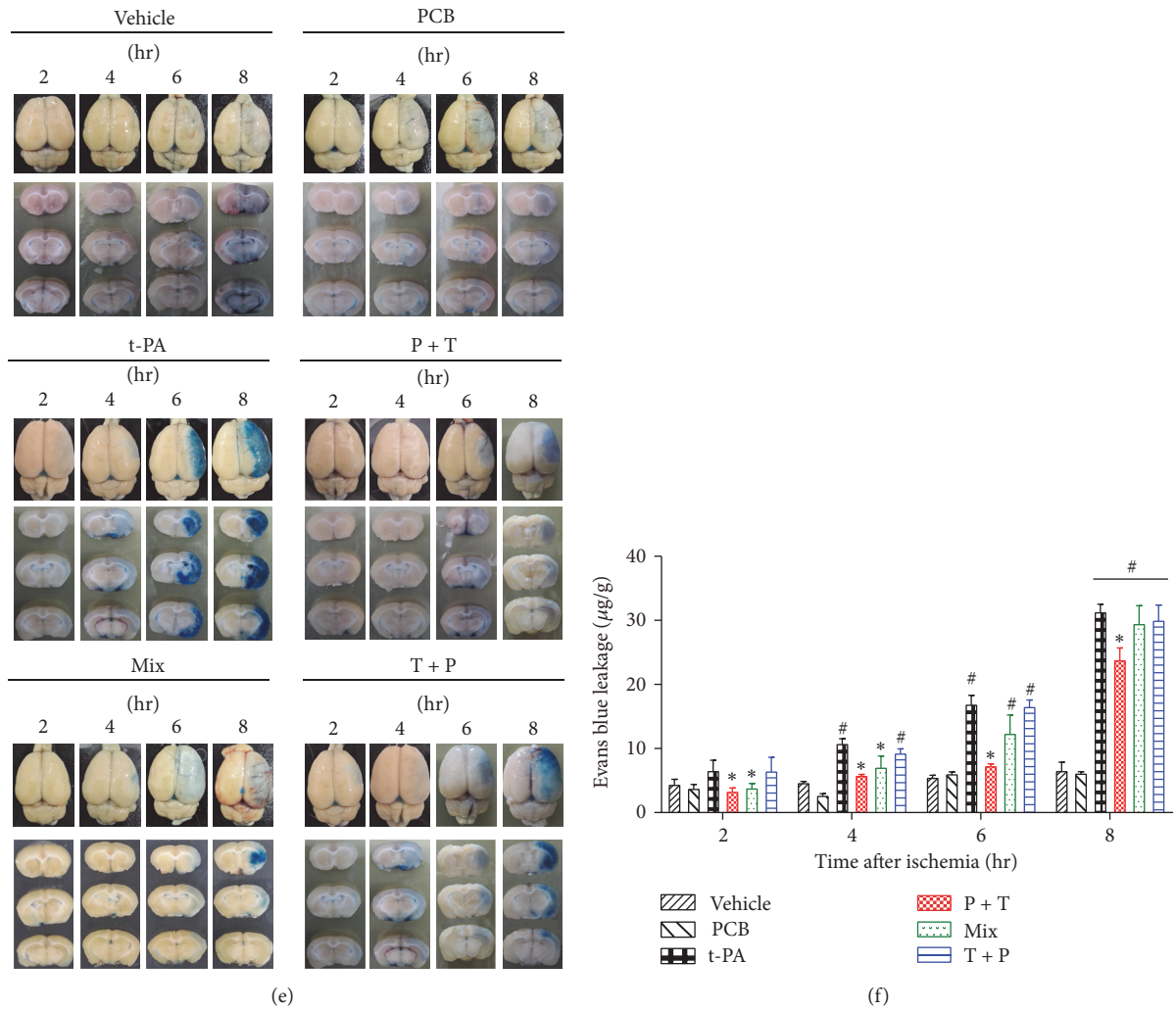

Figure 1: Combination of t-PA and PCB reduces EB leakage in the ischemic brain after 2, 4, 6, or 8 hours of thromboembolic stroke with 2-hour reperfusion. (a) Distribution of PCB in plasma and cerebrospinal fluid (CSF) at 1, 3, 5, 7, and 10 min after the injection. (b) Schematic diagram of the overall experimental design. (c) Focal CBF change during the thrombolysis. Agents were administered at 2, 4, 6, or 8 h after ischemia onset. The time points stand for time (1) before the occlusion, (2) at occlusion, and (3) 2 h after administration. (d) Representative brain slices showing EB leakage in the ischemic tissue. (e) Quantification of EB extravasation is expressed as nanogram per gram of brain tissue (μg/ml). (f) Effect of combination therapy on reducing the brain edema as measured by brain swelling. $^{*}P < 0.05$ versus thromboembolic stroke treated with t-PA; $^{\#}P < 0.05$ versus thromboembolic stroke treated with vehicle saline. Data are expressed as the mean ± SEM ($n = 5$).

rod test, forelimb function test, and inclined plane test were assessed 2 hr after the surgery. The neurological deficits test used a modified 5-point Bederson scale to determine the neurological deficits of the rat [19]. The rotating rod test, forelimb function test, and inclined plane test were modified and used to evaluate hemiparesis. The motor coordination of the forelimbs and hind limbs was assessed as previously reported [20–22].

2.6. Measurement of Evans Blue Leakage. Evans blue (EB; 4% wt/vol in PBS, 2 mL/kg; Sigma) was administered intravenously into the external jugular vein at the onset of reperfusion. At the end of the 2-hour reperfusion, the rats were transcardially perfused with PBS. The brain was then removed, sectioned, and photographed to visualize EB extravasation. We assessed brain edema by measuring hemispheric enlargement. We also quantitatively assessed BBB disruption by measuring EB contents in ischemic hemispheric tissue, as previously reported [23].

2.7. Measurement of Brain Edema. Brain edema was determined by measuring swelling of the ischemic brain tissues. The hemispheric areas of each 2 mm thick brain slice were measured on the digital photographs obtained using ImageJ software (National Institutes of Health), as described previously [24, 25]. Brain swelling was expressed as a ratio of ischemic hemispheric area versus nonischemic hemispheric area.

2.8. Quantification of Infarction Volume. TTC staining was performed at 24 hr after ischemia onset. Rats were euthanized and then beheaded after cervical dislocation. The brains

were perfused with normal saline, and 8 coronal sections (2 mm thick) were stained with 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) and fixed in 4% paraformaldehyde solution. Infarction volume was measured and analyzed with the Image J software. Edema correction was performed as previously reported [26]. After correcting for edema, the volume of infarction is calculated by summing the infarction area from all slices and multiplying the thickness.

2.9. Measurement of MMPs, Occludin, and Claudin-5 in Cerebral Tissue. Brain tissues from the same position as EB measurement were collected after the behavior outcome and edema tests and at 4 hr after ischemia. MMP-2, MMP-9, occludin, and claudin-5 protein levels were measured by Western blot as described [27]. Human MMP-2 and MMP-9 (Chemicon, Temecula, CA, USA) was utilized as the standard (STD) control. All antibodies used in this study were purchased from Cell Signaling Technology, Inc.

2.10. Construction and Characterization of BBB Models In Vitro. For construction of the BBB models, human cerebral microvascular endothelial cells (cerebEND cells) and astrocyte (CTXTNA2) were cultured to confluence on 24-well collagen-coated Transwell™ tissue culture inserts (0.4 μm pore size, Millipore) in 37°C as was demonstrated in Figure 6(b). Cultures were maintained at 37°C and 5% CO_2 in a humidified incubator. The growth medium was changed every day, and the cells were grown to a compact monolayer for about 3 days.

To simulate ischemia-like conditions *in vitro*, cocultures were subjected to oxygen-glucose deprivation (OGD) as described [28]. Briefly, cocultures were exposed to OGD for 6 hours, by replacing the culture medium with a glucose- and serum-free medium that had been equilibrated in an anaerobic atmosphere (at <0.1% O_2, 5% CO_2, and 95% N_2) inside a cell incubator. For the reperfusion, cells are transferred to the normal incubator (95% room air and 5% CO_2) with media change (normal glucose: 5.5 mM) and incubated for another 2 hours. For normoxic controls, cocultures were incubated for 8 hours in glucose-containing, serum-free medium equilibrated with air. BBB permeability was assessed using 10 kDa dextran-conjugated FITC (1 mg/ml) (Sigma-Aldrich) and fluorescence intensity in the lower and upper chamber was measured using a SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA). BBB permeability was calculated as the ratio of lower/upper chamber.

2.11. Statistical Analysis. Data were presented as the mean ± SEM. In most cases, statistical analysis was carried out using a one-way ANOVA, with the post hoc Newman–Keuls analysis. For comparison of EB leakage between ischemic hemispheres and nonischemic hemispheres, a paired t-test was performed. For comparison of the mortality between t-PA and vehicle or the combination therapy, a chi-square (χ^2) test was performed. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Pharmacodynamic Characteristics of PCB Was in Accordance with Its Distribution in CSF. To make sure PCB reached an adequate brain concentration before t-PA administration, we firstly determined the pharmacokinetic changes of PCB in the plasma and cerebrospinal fluid (CSF). We found that PCB quickly disappeared in the plasma after injection and the peak concentration of PCB in CSF appeared at approximately 7 min after injection (Figure 1(a)). Therefore, we decided to inject PCB 5 min before t-PA administration and reperfusion started (P + T) to maximize the potential protective effects of PCB (Figure 1(b)). PCB was also administered together with or right after t-PA infusion as controls (Mix or T + P, resp.; Figure 1(b)).

3.2. PCB Attenuated the Progression of BBB Damage following Prolonged Ischemia. To determine whether PCB could attenuate the progression of ischemic BBB damage caused by delayed thrombolysis, we compared the severity of BBB damage via three different ways of PCB and t-PA coadministration as described above after 2, 4, 6, or 8 hours of ischemia followed by 2-hour reperfusion. Successful occlusion (below 30%) of MCA by thrombus and recanalization (above 70%) by t-PA was confirmed by monitoring the cerebral blood flow via laser Doppler (Figure 1(c)). Figures 1(d)-1(e) shows the EB extravasation after 2, 4, 6, or 8 hours of cerebral ischemia. As expected, EB contents in nonischemic hemispheric tissue were low under all tested stroke conditions. EB content was significantly increased in the ischemic brain tissue and greater EB leakage was seen for longer ischemic duration in both vehicle and t-PA groups. PCB treatment alone did not affect EB extravasation for all 4 ischemic durations. Interestingly, PCB pretreatment 5 min prior to t-PA infusion (P + T) significantly reduced the EB leakage following 2 hr, 4 hr, 6 hr, and even 8 hr ischemia, which was far beyond the 4.5 hr t-PA therapeutic time window, as compared with t-PA infusion alone (Figures 1(d) and 1(e)). Mixed PCB and t-PA administration significantly decreased EB leakage following 2 to 6 hr ischemia as well but was less effective (Figures 1(d) and 1(e)). PCB injection after t-PA infusion appeared to be ineffective.

Brain edema is a major complication of BBB damage. In line with the EB leakage results, t-PA infusion resulted in elevated brain edema as measured by brain swelling after prolonged ischemia (6 hr and 8 hr), which could be significantly inhibited by PCB pretreatment or mixture, but not by PCB posttreatment (Figure 1(f)). These results indicated that t-PA infusion-induced BBB damage and edema occurred rapidly and there was a very short time window for interventional protection of BBB by PCB.

3.3. PCB Improved Short-Term Behavioral Outcomes following Prolonged Ischemia. As to the neurological and behavioral outcomes, we measured the neurologic deficit score, forelimb function, rotating rod test score, and holding time in rats receiving thromboembolic stroke and various treatments. As shown in Figure 2, in rats subjected to 2–8 h of embolism, t-PA infusion alone improved performance at early time points

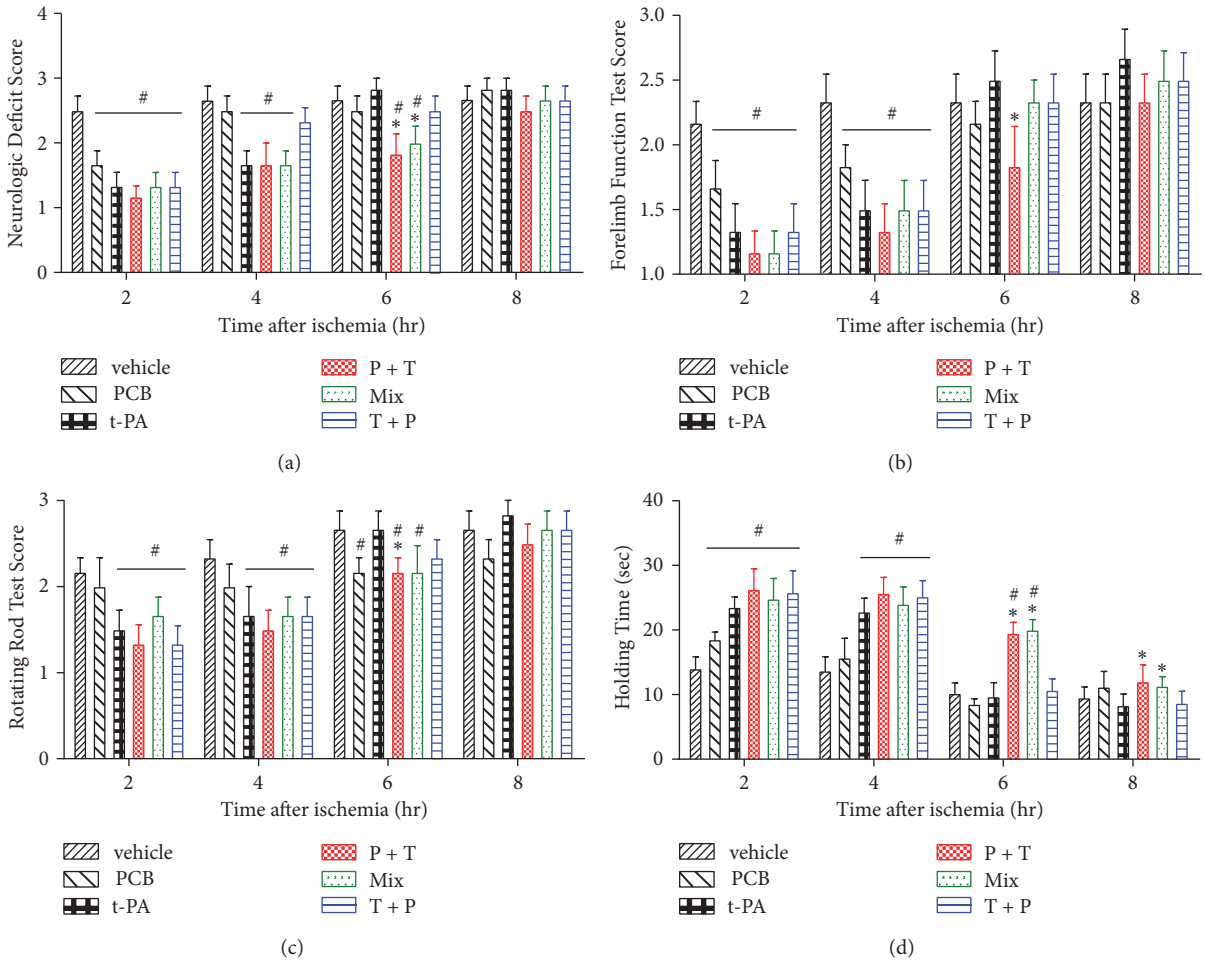

Figure 2: Combination of t-PA and PCB ameliorates behavior outcomes, cerebral edema, and blood supply after 2, 4, 6, or 8 hr of thromboembolic stroke with 2-hour reperfusion. (a)–(d) Neurological deficits test, forelimb function test, rotating rod test, and inclined plane test were examined after 2-hour reperfusion by combination of t-PA and PCB. $^{*}P < 0.05$ versus thromboembolic stroke treated with t-PA; $^{\#}P < 0.05$ versus thromboembolic stroke treated with vehicle saline. Data are expressed as the mean ± SEM ($n = 10$).

(2 hr and 4 hr) but not at late time points (6 hr and 8 hr) in all four tests at 2 h after thrombolytic therapy (Figure 2). However, PCB pretreatment + t-PA infusion significantly improved performance on all behavior tests at the late time points (6 hr and 8 hr), as compared to t-PA alone. The Mix group displayed a less beneficial effect than P + T group but was better than the T + P group after 6 hours' ischemia. Interestingly, PCB treatment alone also improved the behavioral scores of rats after 2 hr and 4 hr ischemia even if it did not induce reperfusion (data not shown), implying PCB possibly reached the ischemic brain tissue through contralateral circulation.

3.4. PCB Pretreatment Reduced Brain Infarction Volume following Prolonged Ischemia. We next conducted TTC staining to evaluate the effect of PCB on brain infarction. Given that PCB pretreatment (P + T) displayed the most pronounced protective effect, we focused on investigating the therapeutic effect of P + T hereafter. The TTC staining results showed that unlike the BBB damage when t-PA was given within the therapeutic time window (2 hr and 4 hr ischemia), t-PA infusion significantly reduced the infarction volume (Figure 3). When t-PA was given beyond the therapeutic time window (6 hr and 8 hr ischemia), t-PA infusion was not able to reduce brain infarction anymore. However, PCB pretreatment prior to t-PA infusion significantly reduced the infarction volume following 6 hr ischemia (Figure 3, $^{*}P < 0.01$). PCB treatment alone also showed protective effect on infarction after 2 hr and 4 hr ischemia, in line with the behavioral test results (Figure 2).

3.5. Repeated PCB Treatment Improved Long-Term Behavioral Outcomes after Delayed t-PA Administration following Prolonged Ischemia. The results above clearly showed that one time PCB pretreatment could attenuate delayed t-PA infusion-induced BBB damage and improved neurological functions beyond the standard 4.5 hr time window. We next wondered whether repeated PCB treatment after the initial PCB + t-PA administration could further improve the long-term behavioral outcomes in ischemic rats. PCB + t-PA was administered after 6 hr ischemia as in Figure 1(b); then PCB was injected every 24 hr for 7 days. The results showed

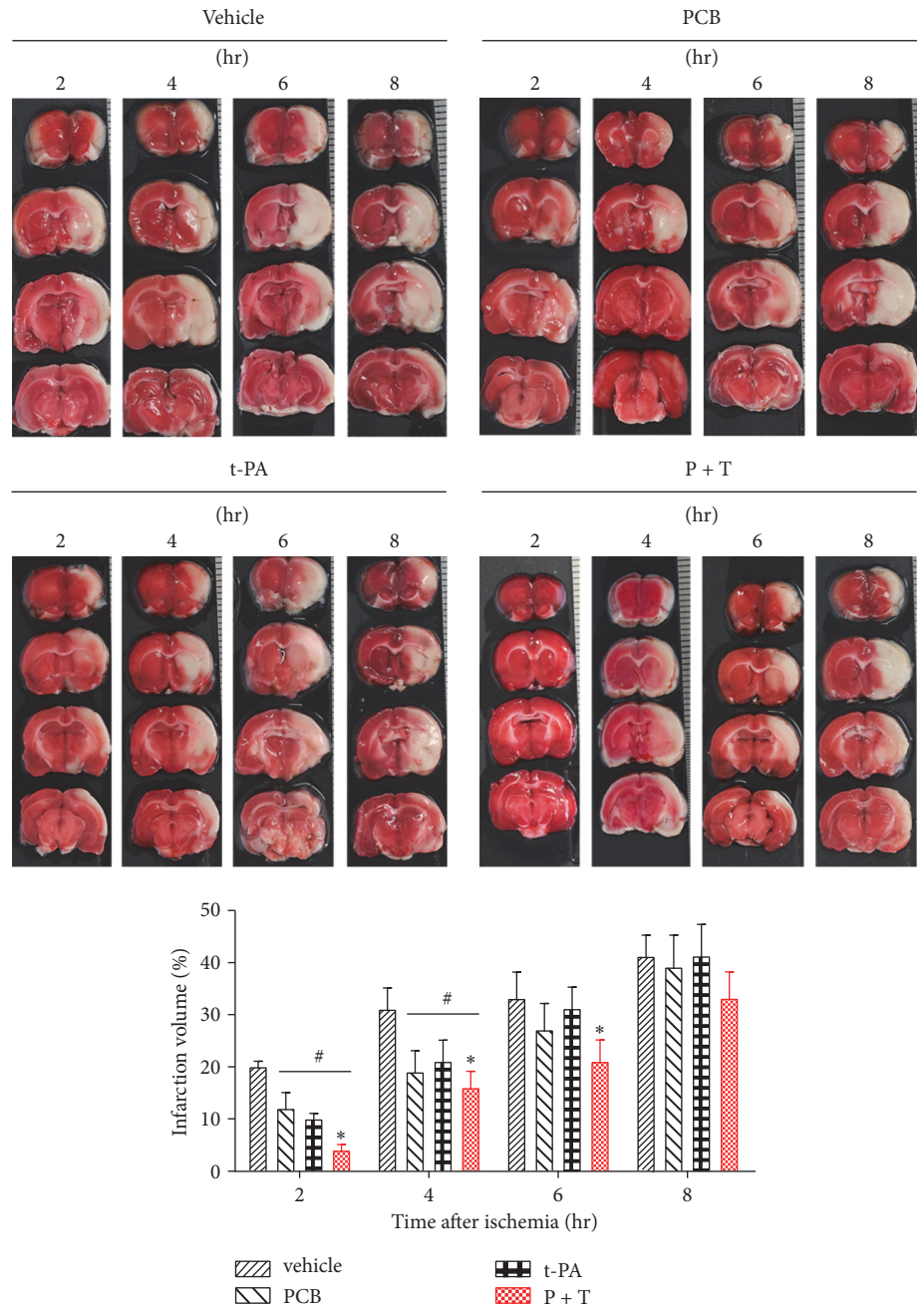

Figure 3: Effect of PCB on the infarction volume. Representative images of TTC-stained brain sections 24 hr after the PCB and/or t-PA therapy. Agents were administered intravenously at 2 hr, 4 hr, 6 hr, or 8 hr after ischemia onset. $^{*}P < 0.05$ versus thromboembolic stroke treated with t-PA; $^{\#}P < 0.05$ versus thromboembolic stroke treated with vehicle saline. Data are expressed as the mean ± SEM ($n = 5$).

that both t-PA infusion alone and repeated PCB treatment alone could modestly improve the long-term behavioral performance (neurologic deficit score, forelimb function, rotating rod test, and holding time; Figures 4(a)–4(d)). Importantly, repeated PCB treatment after the initial P + T coadministration substantially improved the long-term behavioral performance.

In the first two days, a large part of the rats treated with t-PA alone died because of severe hemorrhage and ischemic infarction, consistent with previous reports [29–31]. By contrast, the PCB combination therapy (P + T) animals had a significantly lower mortality as compared with the t-PA group (Figure 4(e)).

As shown in Figure 4(f), rats in the vehicle group displayed a time-dependent body weight loss after thromboembolic stroke with a rebound beginning from day 6. Rats in the t-PA group displayed a similar weight loss, with the lowest weight on days 5 to 6. However, treatment with PCB alone or t-PA + PCB alleviated the weight loss significantly ($^{*}P < 0.05$).

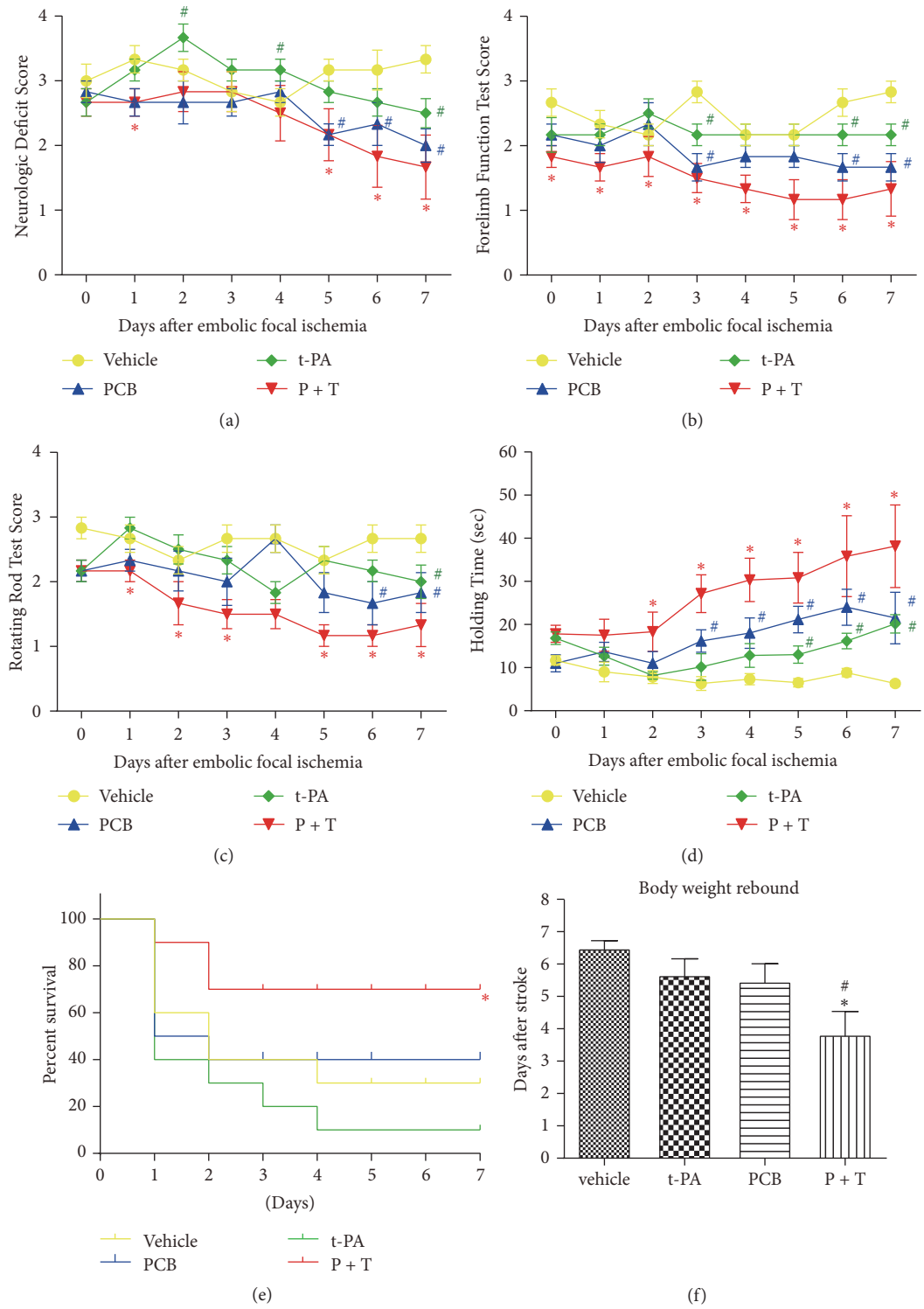

FIGURE 4: Combination of t-PA and PCB improves long-term behavior outcomes. PCB was administrated every 24 hr for 7 d after the combination therapy at 6 hr after thromboembolic stroke. (a) Neurological deficits test, (b) forelimb function test, (c) rotating rod test, and (d) inclined plane test were examined after the combination therapy at 6 hr after thromboembolic stroke in thromboembolic stroke rats. $^{*}P < 0.05$ versus thromboembolic stroke treated with t-PA; $^{\#}P < 0.05$ versus thromboembolic stroke treated with vehicle saline. Data are expressed as the mean ± SEM ($n = 10$). (e) Mortality rates were 70% (7/10) in saline-treated rats and 90% (9/10) in the delayed t-PA treatment group, whereas therapy with PCB + t-PA reduced mortality to 30% (3/10) at 7 d after reperfusion ($n = 10$, $^{*}P < 0.05$ versus t-PA, χ^2 test). (f) Body weights were recorded and the rebound points were compared. $^{*}P < 0.05$ versus thromboembolic stroke treated with t-PA; $^{\#}P < 0.05$ versus thromboembolic stroke treated with vehicle saline. Data are all expressed as the mean ± SEM.

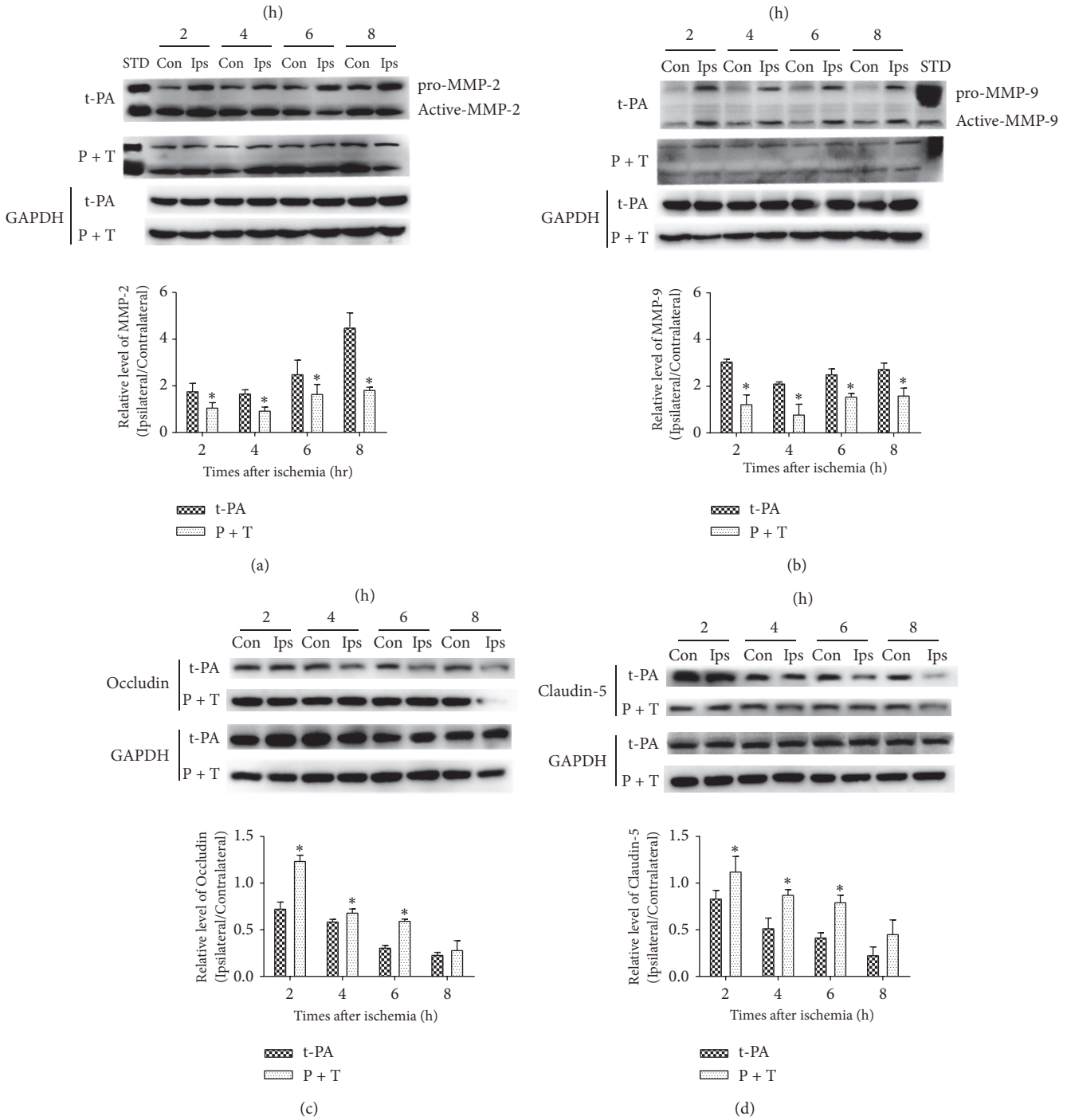

Figure 5: PCB decreased t-PA-induced MMP-2 and MMP-9 activation and TJs (occludin and claudin-5) loss in thromboembolic stroke. ((a) and (b)) the MMP-2 and MMP-9 protein levels in the contralateral (Con) and ipsilateral (Ips) brain tissue in t-PA and PCB + t-PA-treated rats and the quantitative analysis result. STD, the standard of MMP-2 or MMP-9. ((c) and (d)) Western blot analysis of occludin and claudin-5 in cell membrane with different treatments. Rats received t-PA (1 mg/kg) and PCB (10 mg/kg) and tissues were analyzed 4 hr after varied hours of thromboembolic stroke in different groups ($n = 3$; $^*P < 0.05$, versus nonischemic, paired t-test).

3.6. PCB Inhibited Tight Junction Protein (TJP) Loss in Ischemic Tissue after Delayed t-PA Administration. To explore the causes responsible for the beneficial role of PCB, we examined matrix metalloproteinase (MMP) induction and TJP loss under the same experimental regimen as described in Figure 1(b). As shown in Figures 5(a)-5(b), MMP-2 and MMP-9 protein levels were elevated after t-PA administration in ischemic hemisphere as compared to the contralateral hemisphere. Importantly, PCB treatment abolished MMP-2 and MMP-9 induction in the ischemic tissue of t-PA-treated

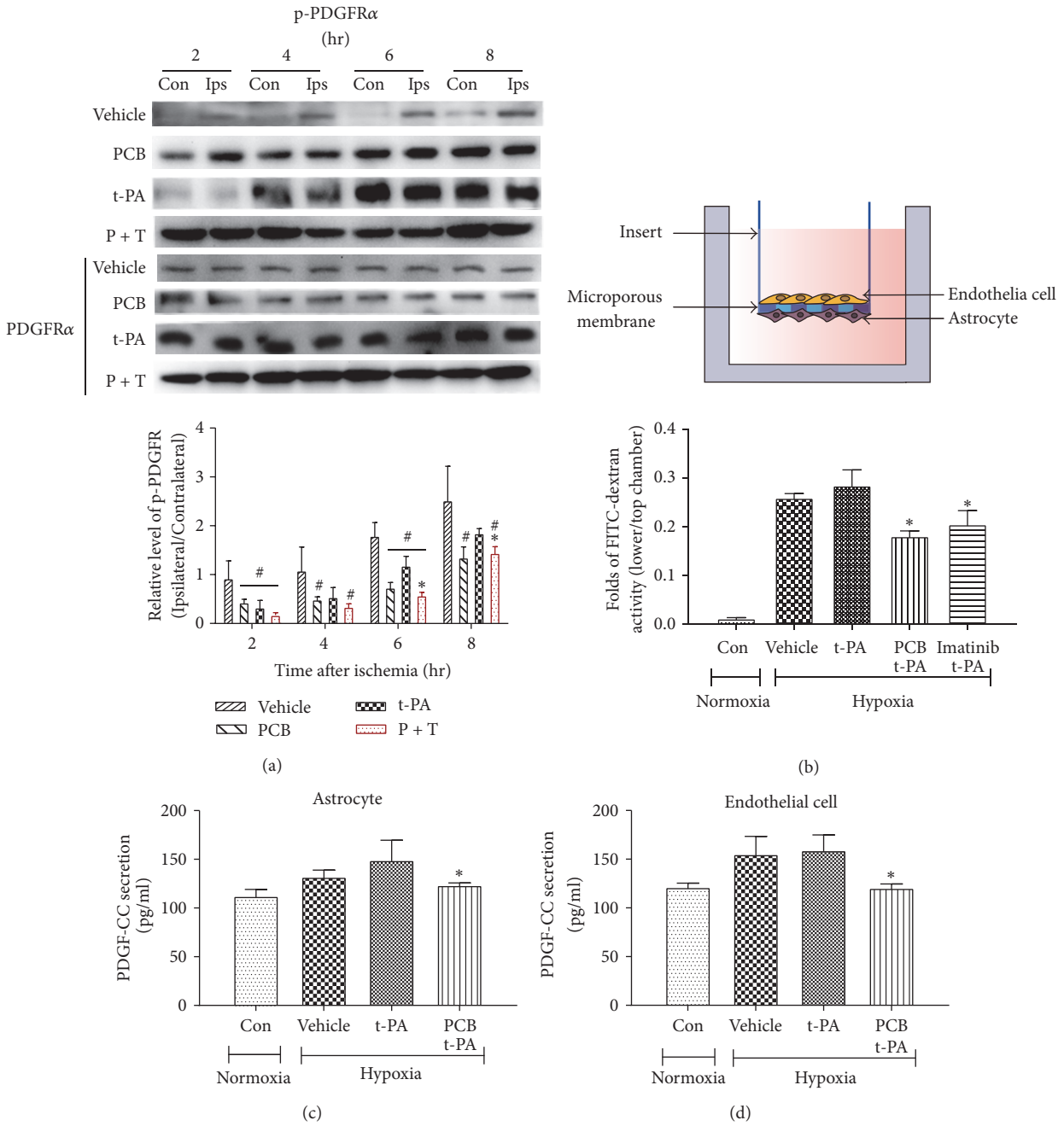

Figure 6: PCB reduced the permeability of BBB model *in vitro* by inhibiting the PDGF-CC/PDGFRα signaling pathway. (a) Western blot analysis of phosphorylated PDGFRα in cell membrane with different treatments. (b) Schematic of BBB model in vitro by using the Transwell insert. The permeability of BBB model was tested during 6 h of hypoxia/aglycemia and 2 h of reperfusion. (c-d) The contents of PDGF-CC in the culture medium of CTXTNA2 and cerebEND cells ($n = 3$; $^{*}P < 0.05$, versus t-PA; $^{\#}P < 0.05$ versus vehicle saline).

rats following 2 to 8 hr ischemia. Accordingly, ischemia caused a time-dependent loss of occludin and claudin-5 in ischemic tissue after t-PA administration (Figure 5(c)). The PCB pretreatment prior to t-PA infusion reduced the loss of occludin and claudin-5 in rats following 2 to 8 hr ischemia. It is worth noting that PCB only treatment minimally affected the above events (data not shown). The above results demonstrated that PCB might suppress t-PA-mediated BBB damage at least in part by decreasing the induction of MMPs and TJP loss.

3.7. PCB Protected BBB Integrity by Inhibiting the PDGF-CC/PDGFRα Signaling Pathway. Previous studies showed that t-PA induces opening of the BBB through activation of the PDGF-CC/PDGFRα pathway [32, 33]. We firstly determined the phosphorylation of PDGFRα from the brain tissue of embolic stroke rats. The results showed that the phosphorylation of PDGFRα in the P + T treatment group was significantly lower than t-PA group (Figure 6(a)). We next utilized an *in vitro* BBB model and oxygen and glucose deprivation (OGD) to mimic the *in vivo* ischemic

stroke condition. We found that after a 6 hr OGD and 2 hr reperfusion the integrity of BBB was compromised, as measured by the fluorescence intensity of FITC-dextran in lower/upper chamber (Figure 6(b)). We found t-PA slightly exacerbated the opening of BBB, whereas PCB or PDGFRα inhibitor Imatinib significantly preserved the BBB integrity in the presence of t-PA under OGD situation. Accordingly, we found that secretions of PDGF-CC from astrocytes and endothelial cells were both decreased significantly in the presence of PCB (Figures 6(c)-6(d)), which explained why PCB acted similarly to Imatinib to preserve BBB function. The data above showed that PCB exerts its protective effect on BBB integrity at least in part through inhibition of the PDGF-CC/PDGFRα pathway.

4. Discussion

In the current study, we comprehensively investigated the protective effects and underlying mechanisms of PCB on t-PA infusion-induced BBB damage in a novel rat thromboembolic stroke model we recently developed. We demonstrated that PCB pretreatment before t-PA administration significantly reduced BBB damage and brain edema and infarction, improved the short-term and long-term behavioral outcomes, and increased survival following 6 hr ischemia, which is beyond the standard 4.5 hr t-PA therapeutic time window. We further showed that PCB preserved BBB integrity by inhibiting degradation of tight junction proteins and activation of the PDGF-CC/PDGFRα pathway. Our study presented a potential effective adjunct therapy to increase the safety and the therapeutic time window of t-PA following ischemic stroke.

Tissue plasminogen activator (t-PA) has been demonstrated to be a successful thrombolytic drug in acute ischemic stroke patients but significantly increases the risk of symptomatic HT, which represents the main limitation for thrombolysis [34, 35]. In the National Institute of Neurological Disorders and Stroke (NINDS) t-PA trial [36], the percentage of t-PA-treated patients who developed significant HT following an ischemic stroke was 6.4% compared with 0.6% in the placebo group.

Thrombolysis of the occluded vessel should rescue the affected ischemic zone and improve clinical outcome. However, administering t-PA beyond the 3.5~4.5 hr time window increases BBB injury and then thrombolytic t-PA crosses to the perivascular tissue and causes hemorrhage by interacting with the neurovascular unit [5].

PCB is emerging as a potent neuroprotective molecule that protects neurovascular unit against ischemic stroke injury attributed to its pleiotropic effects on inflammation, ROS, apoptosis, and mitochondrial function [8–13, 37–39]. For instance, PCB protects primary cultured rat cerebral microvascular endothelial cells from the damage induced by oxygen-glucose deprivation/reoxygenation [38]. Other studies indicated that PCB restored BBB integrity or reduced MMP-9 gene expression [37, 40]. Recent studies showed that PCB could significantly mitigate neuroinflammation through inhibiting TLR4 signaling pathway and M1-like microglial polarization in an intracerebral hemorrhage and traumatic brain injury model [12, 13]. With the increasing use of thrombolysis with t-PA, it is important to know if and how PCB affects cerebral hemorrhage associated with t-PA therapy when used in combination with t-PA.

To address the issue, we used three different coadministration approaches of PCB and t-PA in a thromboembolic stroke rat model to elucidate the most curative effect. Interestingly, PCB slowed the progression of ischemia-induced BBB disruption, thus expanding the therapeutic time window of thrombolysis therapy. Compared to t-PA alone, PCB administered 5 min before t-PA infusion (P + T) led to a significant improvement in behavior outcomes and reductions in brain edema and infarction, despite prolonged ischemia durations (6–8 hr).

In this study, we used a novel thromboembolic stroke model that has been a reliable tool in cerebral thrombolysis research, to mimic the clinical ischemic stroke situation. In accordance with other studies, mild signs of bleeding (small petechiae within the damaged area) appeared within 2–4 hr of ischemia in the vehicle group when measured 2 hr after EB injection [41]. Administration of t-PA 2 and 4 hr after occlusion induced recovery of the cerebral blood flow rate up to 70% of initial values. Additionally, a better stroke outcome was observed, as indicated by reduced brain edema and infarction and better behavior test scores. Prolonged ischemia duration, especially in the t-PA administration group, exhibited remarkable differences versus its early counterpart.

BBB breakdown is a common pathological process that occurs after cerebral ischemia-reperfusion and is thought to be a prerequisite for HT and poor treatment outcomes [42]. MMPs are elevated in ischemic brain tissue and critically contribute to BBB disruption, brain edema formation, and cerebral hemorrhage via proteolytic degradation of BBB structural components, including TJPs [6, 43]. In accordance with other studies, our data showed that protein levels of MMP-2 and MMP-9 were induced in an ischemia time-dependent manner, and this change was accompanied by the loss of TJPs occludin and claudin-5 [17]. Administration of P + T ameliorated MMP-2 and MMP-9 induction and TJP loss.

PDGF-CC is a specific substrate of t-PA that binds the PDGFRα. During cerebral ischemia, neuronal depolarization can result in a surge of local and exogenous t-PA activity, which in turn leads to continued production of PDGF-CC, persistent activation of PDGFRα in the neurovascular unit, and, ultimately, loss of BBB integrity. In our study, we found that P + T treatment inhibited the phosphorylation of PDGFRα in ischemic brain tissue compared to t-PA infusion. PCB treatment *in vitro* reduced the secretion of PDGF-CC in astrocytes and endothelial cells under OGD situation, implying a mechanism of how PCB inhibited the PDGF-CC/PDGFRα signaling. However, the current limitation is that we still could not locate the direct target of PCB. Future work is warranted to clarify the exact mechanism of how PCB preserves the BBB integrity.

In conclusion, our study provided direct evidence that administration of PCB 5 min before t-PA infusion reduced cerebrovascular permeability and stroke lesion volume as well as neurologic deficits and increased mortality associated with late thrombolysis. PCB could be a potential novel therapy to

enhance the safety of t-PA thrombolysis following prolonged ischemic stroke.

Disclosure

The mentioned funders were not involved in the study design, data collection, data analysis, manuscript preparation, and/or publication decisions.

Conflicts of Interest

There are no conflicts of interest in this study.

Acknowledgments

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Research Article

Compromised Dynamic Cerebral Autoregulation in Patients with Epilepsy

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Objective. The aim of this study is to analyze dynamic cerebral autoregulation (dCA) in patients with epilepsy. *Methods.* One hundred patients with epilepsy and 100 age- and sex-matched healthy controls were recruited. Noninvasive continuous cerebral blood flow velocity of the bilateral middle artery and arterial blood pressure were recorded. Transfer function analyses were used to analyze the autoregulatory parameters (phase difference and gain). *Results.* The overall phase difference of patients with epilepsy was significantly lower than that of the healthy control group ($p = 0.046$). Furthermore, patients with interictal slow wave had significant lower phase difference than the slow-wave-free patients ($p = 0.012$). There was no difference in overall phase between focal discharges and multifocal discharges in patients with epilepsy. Simultaneously, there was no difference in mean phase between the affected and unaffected hemispheres in patients with unilateral discharges. In particular, interictal slow wave was an independent factor that influenced phase difference in patients with epilepsy ($p = 0.016$). *Conclusions.* Our study documented that dCA is impaired in patients with epilepsy, especially in those with interictal slow wave. The impairment of dCA occurs irrespective of the discharge location and type. Interictal slow wave is an independent factor to predict impaired dCA in patients with epilepsy. *Clinical Trial Identifier*. This trial is registered with NCT02775682.

1. Introduction

The relationship between epileptic seizures and stroke is intimate but complex. Epilepsy not only is a common neurologic sequela of stroke, but also could sometimes herald a stroke [1, 2]. Since the first epilepsy preceding stroke was described in early 1982 [3], postepilepsy stroke had been noted. Simultaneously, several studies reported that patients with epilepsy tend to have a higher stroke risk [4–6]. The potential mechanisms remain unclear. Studies have reported both supranormal demands of cerebral blood flow and disruption of neurovascular coupling after epileptic discharge [7, 8]. These abnormalities in cerebral hemodynamics could well be an important mechanism in the development of postepilepsy stroke.

Dynamic cerebral autoregulation, a mechanism to maintain cerebral blood flow, is a reliable method to evaluate cerebrovascular function and has been proven to be critical for the occurrence [9], development, and prognosis [10] of ischemic stroke. Because seizure has potential effect on cerebral hemodynamics, cerebral autoregulation may be of particular importance to patients with epilepsy to maintain stable cerebral blood flow. It has been demonstrated in animals that cerebral autoregulation is disrupted during both seizures and the subsequent postictal state, and impaired cerebral autoregulation may be involved in the pathogenesis of ischemic brain lesions [11, 12]. However, few studies with limited numbers have examined cerebral autoregulation in humans with epilepsy to date.

In the study, we hypothesize that cerebral autoregulation is impaired in patients with epilepsy during the interictal state, which has a role in the occurrence of postepilepsy stroke. We use transfer function method, the most commonly used method to quantify dynamic cerebral autoregulation [13] based on spontaneous fluctuations of blood pressure and cerebral blood flow velocity, to identify our hypothesis. If this assumption is valid, cerebral autoregulation may become a potential intervention target for preventing postepilepsy stroke.

2. Materials and Methods

The prospective study design was approved by the ethics committee of the First Hospital of Jilin University under the guidelines of the Declaration of Helsinki (1964). All the participants/guardians signed the written informed consent forms. The study is listed at clinicaltrials.gov/under identifier NCT02775682.

2.1. Participants. Patients with epilepsy who were already scheduled for EEG examination were recruited from the Department of Neurology, First Hospital of Jilin University, between April 2016 and May 2017. Each patient received a diagnosis of epilepsy by two experienced neurological physicians separately according to the operational clinical definition of epilepsy recommended by the International League Against Epilepsy (ILAE) in 2013 [14]. All patients underwent CT/MRI examination when first diagnosed with epilepsy. We placed no restriction on age and sex. The exclusion criteria included (1) patients with status epilepticus; (2) intracranial and/or extracranial major vascular stenosis/occlusion diagnosed by a transcranial Doppler (EMS-9PB, Delica, China) and carotid ultrasound (IU22, Phillips, Andover, MA), based on the criteria defined by Wong et al. [15]; (3) a prior symptomatic cerebral vascular disease; (4) a history of brain trauma, brain tumor, encephalitis, and other symptomatic neurological diseases; (5) a history of arterial hypertension, cardiovascular disease, diabetes, hyperlipidemia, current arrhythmia, hyperthyroidism, and anemia, which may undermine hemodynamic stability, or inability to cooperate sufficiently to complete the cerebral autoregulation examination; (6) insufficient bilateral temporal bone windows for insonation of the middle cerebral artery; and (7) intolerance to cerebral autoregulation measurements. Age- and sex-matched healthy controls without epilepsy were recruited from the same region who otherwise met the same eligibility criteria as the patients.

2.2. Dynamic Cerebral Autoregulation Measurement. The procedures of dynamic cerebral autoregulation assessment were performed on the basis of the white paper published in 2016 by the International Cerebral Autoregulation Research Network [16]. Continuous cerebral blood flow velocity was recorded noninvasively using transcranial cerebral Doppler device (MultiDop X2, DWL, Sipplingen, Germany) in bilateral middle cerebral artery at a depth of 45 to 60 mm. Spontaneous arterial blood pressure was recorded using a servo-controlled plethysmograph (Finometer PRO, Netherlands) on the subject's middle finger of the right hand positioned at heart level. The analog output of arterial blood pressure was plugged into the transcranial cerebral Doppler device where two channels of cerebral blood flow velocity (bilateral) and the signal of arterial blood pressure were recorded simultaneously. In order to confirm stability of respiration, end-tidal CO_2 was monitored using a capnograph with a facemask attached to the nasal cannula.

In the group of patients with epilepsy, dynamic cerebral autoregulation measurement was performed after EEG examination was completed. All the participants were accessed during 8 to 11 am to minimize the diurnal variation of dynamic cerebral autoregulation. The participants were told to avoid alcohol intake and exercise for at least 12 hours. Caffeinated drinks and the ingestion of a heavy meal were also abstained from for a minimum of 4 hours. The measurement was performed in a quiet, dedicated research laboratory with minimized visual or acoustic stimulation, at a controlled temperature of 22 to 24°C. First, the subjects were told to breathe normally in a supine position for 15 min to measure baseline arterial blood pressure (Omron 711) and heart rates. Then, both cerebral blood flow velocity and arterial blood pressure were recorded simultaneously for 10 min. All the measurements were performed by one experienced operator.

2.3. Data Analysis. Recorded data were processed using MATLAB software (Math Works, Natick, MA, USA). The raw waveforms were sampled at 100 Hz for both arterial blood pressure and cerebral blood flow velocity. Alignment of the raw waveforms was achieved using a cross-correlation function to remove possible time lags caused between the devices. Mean values of the signals within each cardiac cycle were calculated and interpolated by third-order polynomial spline to achieve beat-to-beat signals with a uniform sampling rate at 10 Hz. A third-order Butterworth low-pass filter (cutoff at 0.5 Hz) was then applied as an antialias filter before downsampling the data to 1 Hz. Dynamic cerebral autoregulation was evaluated using transfer function analysis [16, 17]. Fast Fourier transform was used to transform time series of blood pressure and cerebral blood flow velocity to the frequency domain. The transfer function between arterial blood pressure and cerebral blood flow velocity was calculated as the quotient of the cross-spectrum of the two signals and the autospectrum of arterial blood pressure in the low frequency domain (0.06–0.12 Hz) to obtain frequency-dependent estimates of phase difference and gain, where the derived parameters are considered most relevant to autoregulation hemodynamics [18]. At the same time, coherence was calculated to estimate the reliability of the relationship between the two signals at the frequency domain, and the later statistical analysis was performed only if coherence of the parameters was >0.5 [19].

2.4. Statistical Analysis. Continuous variables with a normal distribution, including phase difference and gain, were expressed as mean (standard deviation), while variables with skewed distribution were expressed as median (interquartile range). Discrete variables were expressed as absolute values and percentages. The intergroup differences were tested using

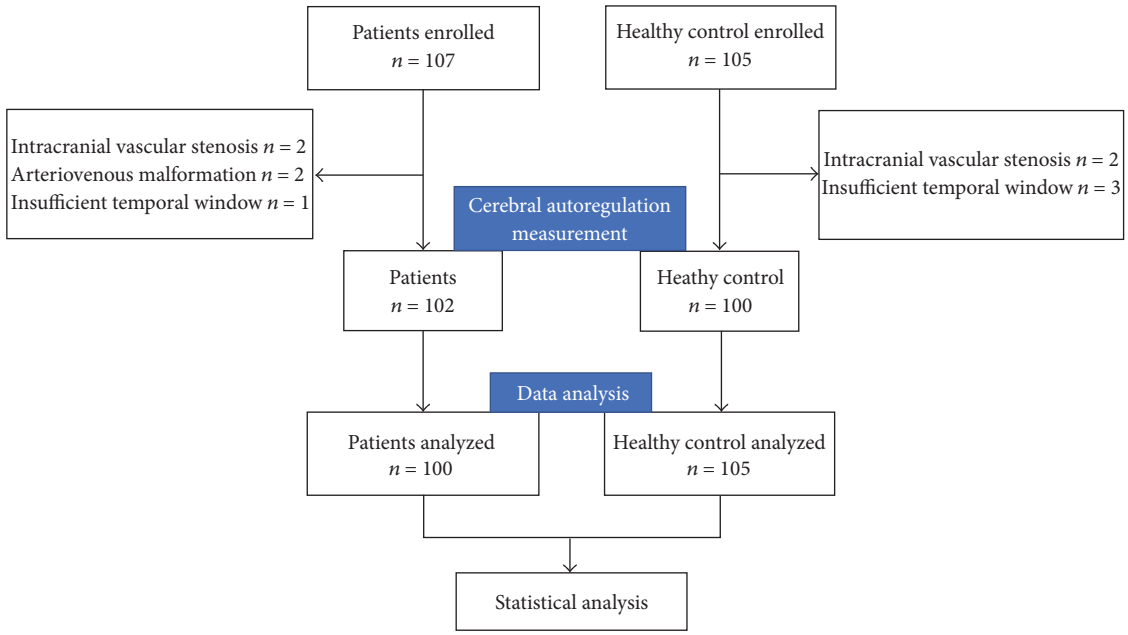

Figure 1: Participant enrollment.

the t-test. We defined the overall phase difference/gain as mean phase difference/gain of bilateral cerebral hemispheres. Univariate and multivariate linear regression were used to assess the association of dynamic cerebral autoregulation parameters and clinical parameters including sex, age, discharge types (focal discharges and multifocal discharges), duration (years), discharge period (discharge at waking versus at sleep state), interictal slow wave, and antiepileptic drugs therapy. All the data were analyzed using the Statistical Program for Social Sciences version 23.0 (SPSS, IBM, West Grove, PA, USA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participant Characteristics. Of 107 patients with epilepsy and 105 age- and sex-matched healthy controls enrolled in this study, 100 patients with epilepsy and 100 healthy controls completed the cerebral autoregulation measurement (Figure 1). Among the patients with epilepsy, 44 (44%) patients had focal discharges (discharge originated from a single site) and 56 (56%) patients had multifocal discharges (discharge originated from more than one lobe or within same lobe of bilateral cerebral but appeared nonsynchronously). The media seizure duration was 6.6 (1,10) years. Fourteen subjects were with new-onset epilepsy. Sixteen patients had epileptic discharges combined with interictal slow wave, and 38 (38%) patients had only epileptic discharges during sleep. Among 66 patients with temporal region seizures, 31 (47%) patients had unilateral discharges, and 35 (53%) patients had bilateral discharges. No patients had a clinical seizure onset during the dynamic cerebral autoregulation measurement. Neurologic examinations were normal in all participants. We did not find any significant differences in sex, age, mean blood pressure, heart rate, or end-tidal carbon dioxide between patients with epilepsy and those in the control group. The clinical characteristics of participants are shown in Table 1.

3.2. Autoregulatory Parameters between Patients with Epilepsy and the Control Group. The overall phase difference of patients with epilepsy was significantly lower than that of the control group (50.20 ± 16.28 versus 54.23 ± 11.84 degree, 95% confidence interval [CI] −8.01 to −0.07, $p = 0.046$), as shown in Figures 2(a) and 2(b). There were no significant differences between the overall gain of the two groups (0.87 ± 0.30 versus 0.84 ± 0.25 cm/s/mmHg, 95% CI −0.05 to 0.11, $p = 0.415$).

Interestingly, patients with interictal slow wave ($n = 16$) had significant lower phase difference than slow-wave-free patients ($n = 84$) (41.11 ± 14.23 versus 51.93 ± 16.14 degree, 95% CI −19.03 to 2.60, $p = 0.012$; Figures 2(c) and 2(d)). The overall gain of these two groups had no significant differences (0.92 ± 0.34 versus 0.86 ± 0.29 cm/s/mmHg, 95% CI −0.13 to 0.24, $p = 0.534$).

3.3. Autoregulatory Parameters of Different Groups of Patients. No significant differences of the dynamic cerebral autoregulation parameters were noted between patients with focal discharges ($n = 44$) and patients with multifocal discharges ($n = 56$) (phase difference 48.16 ± 18.52 versus 51.80 ± 14.25 degree, 95% CI −10.36 to 3.10, $p = 0.286$; gain 0.86 ± 0.30 versus 0.88 ± 0.30 cm/s/mmHg, 95% CI −0.14 to 0.10, $p = 0.757$; Figure 3(a)).

Among patients with temporal region seizure, no significant differences of the overall phase and gain between unilateral discharges ($n = 31$) and bilateral discharges ($n = 35$) were observed (phase difference 48.98 ± 18.10 versus 50.36 ± 16.42 degree, 95% CI −7.11 to 9.86, $p = 0.749$; gain 0.90 ± 0.28 versus 0.87 ± 0.38 cm/s/mmHg, 95% CI

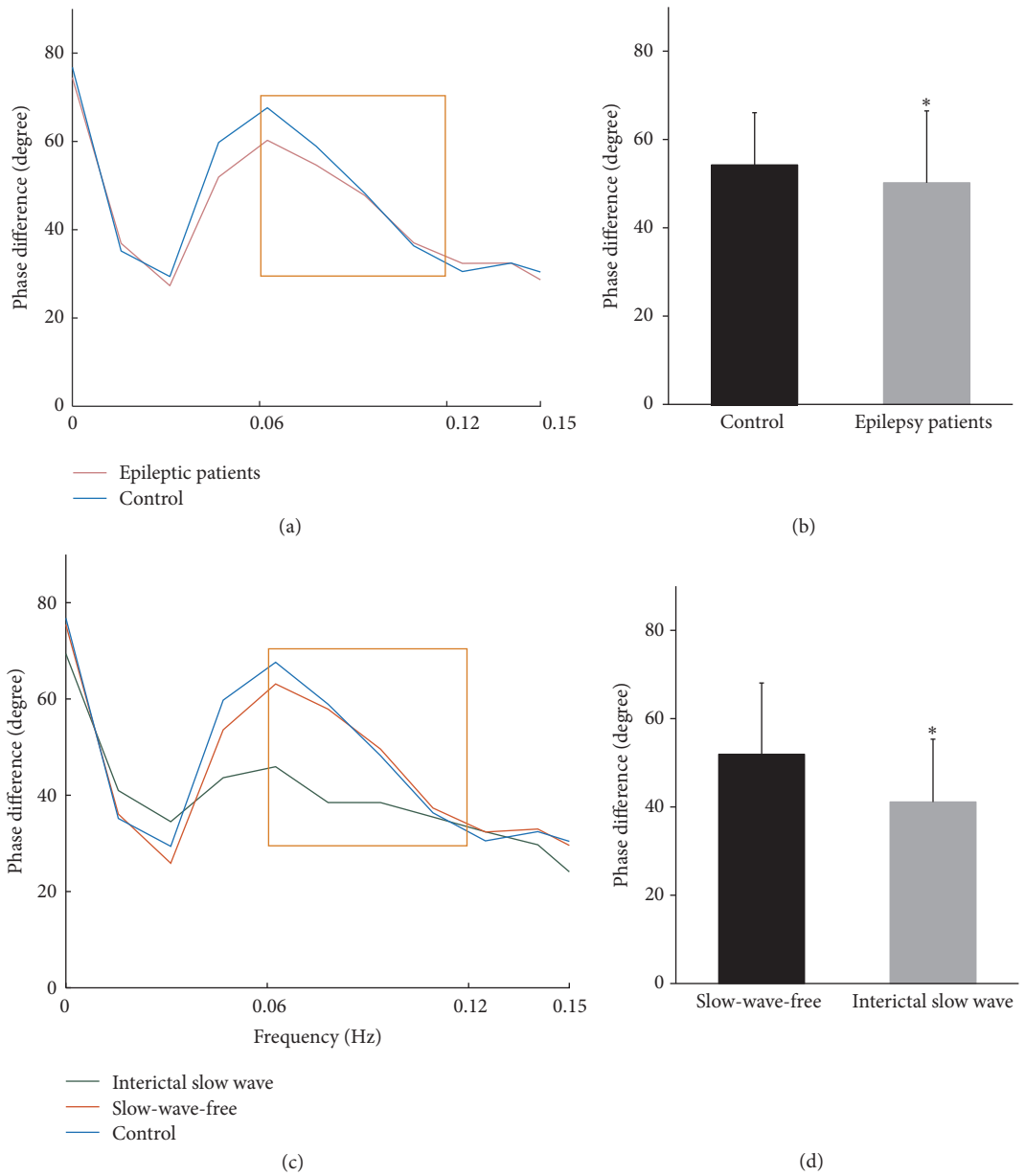

FIGURE 2: *The autoregulatory parameter and statistical distributions in overall epileptic patients and epileptic patients with/without slow wave.* (a) The autoregulatory parameter (phase difference) derived from the transfer function in overall epileptic patients. (b) Statistical distributions of the phase difference in overall epileptic patients. (c) The phase difference derived from the transfer function in epileptic patients with/without slow wave. (d) Statistical distributions of the phase difference in epileptic patients with/without slow wave. In (a) and (c), frames in orange represent specific frequency domain (0.06–0.12 Hz). In (b) and (d), bars denote means; whiskers denote standard error. $N = 16$ for interictal slow wave patients; $n = 84$ for slow-wave-free patients. $*$ indicates statistically different ($p < 0.05$).

−0.19 to 0.14, $p = 0.753$; Figure 3(b)). To identify whether epileptic discharges could influence cerebral autoregulation in the contralateral hemisphere, we analyzed 36 patients with unilateral discharges and did not find significant differences of the two autoregulatory parameters between ipsilateral side and contralateral side (phase difference 49.62 ± 18.21 versus 47.88 ± 18.43 degree, 95% CI −4.63 to 8.10, $p = 0.584$; gain 0.89 ± 0.27 versus 0.88 ± 0.31 cm/s/mmHg, 95% CI −0.09 to 0.09, $p = 0.995$; Figure 3(c)).

3.4. Univariable and Multivariable Analyses. The clinical parameters used in the univariable and multivariable analysis are shown in Table 2. In the univariable model, interictal slow wave was related to lower phase difference ($p = 0.016$). The multivariable model included gender, age, and interictal slow wave. Interictal slow wave was an independent factor that influenced phase difference. No factors were detected associated with gain after multivariable analysis.

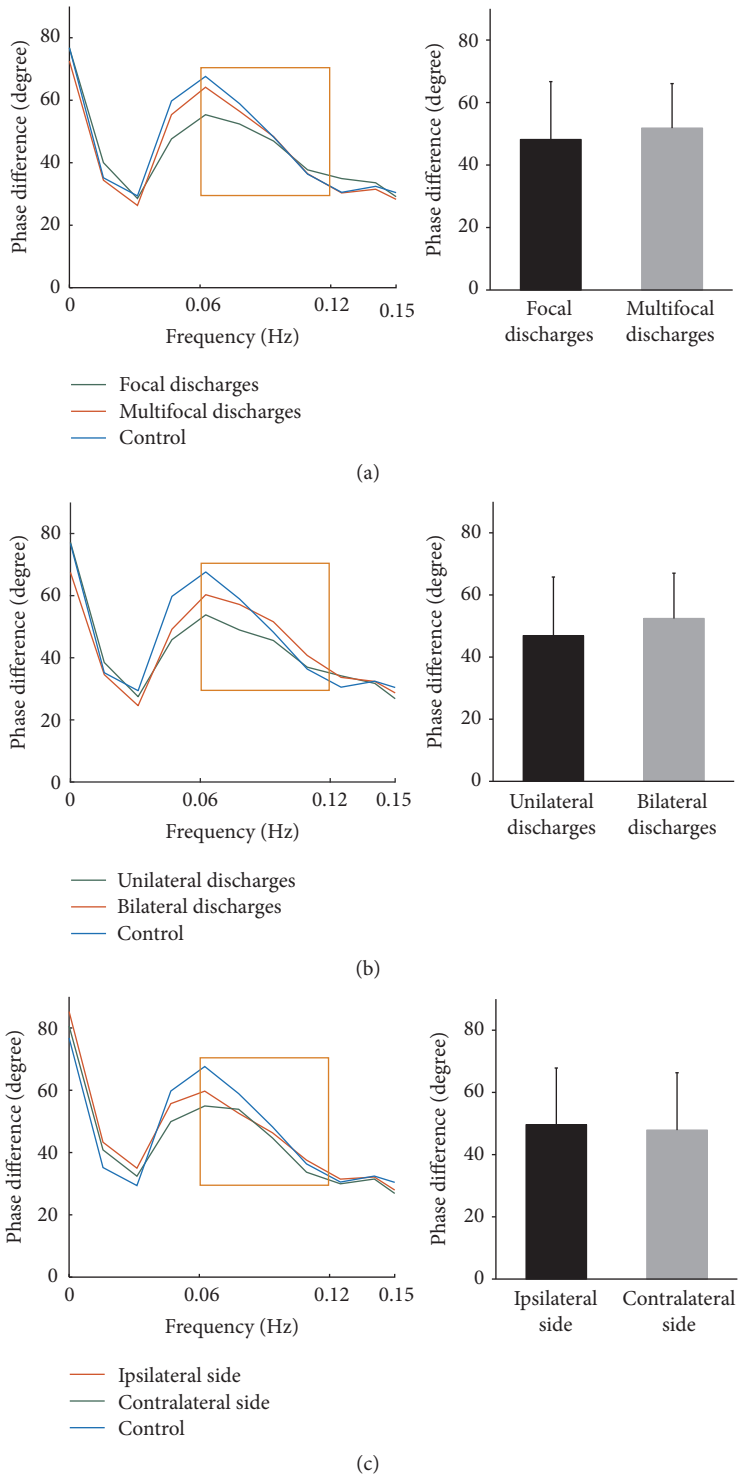

Figure 3: *The autoregulatory parameter and statistical distributions in each group*. (a) Phase difference derived from the transfer function (left) and its statistical distributions (right) in patients with focal discharges and multifocal discharges. (b) Among patients with temporal seizure, the overall phase difference (left) and its statistical distributions (right) in patients with unilateral discharges and bilateral discharges. (c) Mean phase difference (left) and its statistical distributions (right) of ipsilateral side and contralateral side in patients with unilateral discharges. Denotations by lines and frames are similar to those in Figure 2. $N = 44$ for patients with focal discharges; $n = 56$ for patients with multifocal discharges; among 66 patients with temporal seizure, $n = 31$ for unilateral discharges and $n = 35$ for bilateral discharges; $n = 36$ for patients with unilateral discharges.

Table 1: Baseline characteristics of patients with epilepsy and the control group.

	Patients ($n = 100$)	Control group ($n = 100$)	p
Age (year)	32.7 ± 11.8	32.1 ± 10.3	0.703
Gender, male/female	40/60	40/60	1
Smoking, n (%)	17 (17.0%)	10 (10.0%)	0.092
Mean blood pressure (mmHg)	87.3 ± 8.5	86.4 ± 7.5	0.399
Mean MCA velocity	67.69 ± 12.34	64.82 ± 12.41	0.102
Heart rate (beats/min)	70.7 ± 8.1	70.4 ± 7.6	0.773
End-tidal CO_2 (mmHg)	37.6 ± 1.7	37.1 ± 1.8	0.527
Discharge types			
Focal discharges, n (%)	44 (44)		
Multifocal discharges, n (%)	56 (56)		
Epileptic discharge sites			
Temporal region, n (%)	66 (66)		
Frontal region, n (%)	13 (13)		
Multiple regions, n (%)	21 (21)		
Discharge state			
Wake, n (%)	62 (62)		
Sleep, n (%)	38 (38)		
Interictal EEG discharge wave			
Sharp waves, n (%)	40 (40)		
Sharp-wave complex, n (%)	73 (73)		
Spikes, n (%)	12 (12)		
Spike-wave complex, n (%)	20 (20)		
Slow waves, n (%)	16 (16)		
AED therapy, n (%)	48 (48)		

MCA, middle cerebral artery; AED, antiepileptic drugs.

Table 2: Univariable and multivariable analysis of clinical parameters associated with autoregulatory parameters.

	Univariable analysis				Multivariable analysis			
	Phase difference		Gain		Phase difference		Gain	
	β	p	β	p	β	p	β	p
Gender	0.004	0.971	0.037	0.713	0.042	0.681	0.078	0.491
Age	−0.030	0.768	−0.137	0.175	−0.010	0.917	−0.196	0.065
Smoking	−0.108	0.291	−0.101	0.324				
Epileptic discharge sites	−0.056	0.624	−0.054	0.596				
Interictal slow wave	−0.241	0.016[ab]	0.069	0.496	−0.249	0.016[bc]		
Duration	0.167	0.108	−0.154	0.139				
Epileptic discharge type	0.101	0.318	0.034	0.736				
Discharge state	0.006	0.953	−0.011	0.914				
AED therapy	−0.017	0.875	0.127	0.231				

[a]Nominally significant values ($p < 0.1$) included in the multivariable model; [b]p value < 0.05 (statistically different); [c]independent factor that influences cerebral autoregulation.

4. Discussion

In this study, we found the autoregulatory parameter, phase difference, was impaired in patients with epilepsy. There were no differences of the impairment between focal discharges and multifocal discharges and no differences between affected and unaffected hemispheres. In particular, interictal slow wave was an independent factor that influenced phase difference values in patients with epilepsy. These findings may increase understanding of the underlying mechanism

where patients with epilepsy tend to have higher risk of stroke and provide a potential intervention target to prevent postepilepsy stroke.

The first case of epilepsy preceding stroke was described in early 1982 by Barolin [3]. Several years later, a case-control study by Shinton and colleagues showed that preexisting epilepsy was more common in the stroke group, which suggested that epilepsy could sometimes herald a stroke [1]. Subsequently, studies with larger amount of patients supported Shinton's hypothesis in both elderly and young patients [4]. Recently, a prospective study by Sillanpää et al. with five decades of follow-up showed that patients with childhood-onset epilepsy had higher MRI abnormalities, including those with epilepsy in remission, which may be a predictor of clinically evident stroke [20]. The potential mechanisms underlying this phenomenon remain unclear. Except for the use of antiepileptic drugs and the lifestyles of patients with epilepsy that have been demonstrated to be risk factors for stroke (such as smoking, physical inactivity, and certain unhealthy diet choice), epileptic seizures were thought to be an essential cause [21]. Shinton et al. detected that, among the patients who had partial motor epilepsy, a hemiplegia developed on the same side as the epileptic focus in most cases [1]. It was probably the earliest evidence of this kind. Olesen et al. reported a higher risk of stroke in patients with untreated epilepsy [22]. Another study showed the risk of stroke was higher in patients with epilepsy without any vascular risk factors (such as hypertension, atrial fibrillation, cardiovascular disease, diabetes, or hyperlipidemia) [5]. In this study, none of the participants had any identified vascular risk factors, and we did not find any significant influences of clinical factors on dynamic cerebral autoregulation parameters containing smoking and antiepileptic drugs therapy. Because of the altered hemodynamics and hypoperfusion during both ictal and interictal events [7], we speculated that disrupted cerebral hemodynamics caused by epileptic discharges participated in the subsequent stroke.

It is widely accepted that during epileptic seizures, the energy metabolism of the cerebrum increases accompanied by the rising neuronal activity, leading to an increase in cerebral blood flow simultaneously. Studies accessed by fMRI and SPECT have confirmed this [23, 24]. However, several studies have shown the opposite cerebral blood flow changes [25, 26]. For example, frontal region hypoperfusion has been documented in patients with temporal seizures [27, 28]. As the intrinsic mechanism to maintain cerebral perfusion, cerebral autoregulation dilates arterioles to increase cerebral blood flow in the ictal and postictal state [7]. Cerebral blood flow changes underlie an exhaustion of cerebral autoregulation capability [25]. Several decades ago Hascoet et al. demonstrated in animals that autoregulation of cerebral blood flow was impaired during both seizures and the subsequent postictal state [12]. They thought this persistent impaired cerebral autoregulation was relevant in the pathogenesis of hemorrhagic or ischemic brain lesions. However, in Hascoet et al.'s study, newborn piglets were studied at 20 to 90 min after cessation of seizures, and the correlations between cerebral blood flow and mean arterial pressure in this period represented cerebral autoregulation in the postictal state. Cerebral autoregulation during subclinical onset and interictal period has not been described yet. Our study focuses on the interictal period, and compromised cerebral autoregulation during this period suggested that it was not only clinical seizure onset but also interictal epileptic discharges that influenced cerebral hemodynamics. Patients with lower cerebral autoregulation may be prone to hypoperfusion during epileptic events. In addition, we did not find significant differences in dynamic cerebral autoregulation parameters between patients with focal discharges and multifocal discharges, nor did we find the ipsilateral side and contralateral side in patients with unilateral discharges. That is, the cerebral autoregulation of epilepsy patients was impaired bilaterally despite the discharge location and type, suggesting the possibility of distant changes induced by chronic epileptic discharges. Analogously, a study by Dütsch and colleagues demonstrated that temporal lobe epilepsy surgery improved the dynamic cerebral autoregulation parameters gain and phase bilaterally, regardless of the side of surgery. They thought a decrease in interictal epileptic activity mostly led to a decreased sympathetic cerebrovascular modulation after the surgery and thus improved cerebral autoregulation capability [29].

The potential mechanisms of impaired cerebral autoregulation in the interictal period are unclear. Since none of our patients had clinical seizure onset before and during measurement, we believe that repetitive interictal epileptic discharges as well as their underlying etiology influenced cerebrovascular function through a series of neuroendocrine mechanisms, thus affecting cerebral autoregulation. Our hypothesis has some evidence. Above all, despite the controversy, cerebral hemodynamic alterations were detected during variable epileptic discharges [23, 27, 28]. Further, epileptic seizures have been proven to disrupt the neurovascular coupling [7], which is another distinct mechanism to regulate cerebral blood flow. This suggested that epileptic seizures not only cause cerebral hemodynamics alteration but also influence cerebrovascular function. The research of Gómez-Gonzalo et al. showed that astrocyte activation resulted from Ca^{2+} elevation and participated in the control of neurovascular coupling and vasomotor response during epileptic activity. However, they observed that compared with the ictal discharges the efficacy of interictal discharges was too poor to elicit cerebral arteriole response, which differed from our findings [30]. In our opinion, as Ca^{2+} elevation and isolated astrocytes activation were indeed seen during interictal discharges, it was likely that chronic and repetitive interictal discharges elicited neurovascular coupling alteration and cerebrovascular dysfunction combined with occasional ictal events. Moreover, Rosengarten et al. observed that neurovascular coupling might have identical mechanisms with cerebral autoregulation [31], which helped explain our results. In addition, interictal autonomic nervous system dysfunction was seen in patients with epilepsy [32, 33], which affected the cerebral autoregulation [34, 35]. Autonomic dysfunction was thought to accelerate with duration and depends on the degree of seizure control [36]. Similarly, Dütsch et al. considered that enhanced autonomic cerebrovascular

function could explain the cerebral autoregulation recovery after surgery in patients with temporal lobe epilepsy [29].

Another important finding of this study was that slow wave was an independent factor that influenced phase difference in patients with epilepsy. Slow wave is a fundamental cortical rhythm that generally emerges in deep nonrapid eye movement sleep [37], mainly composed of delta slowing. In the waking state, slow wave is produced by lesions in both cortical gray matter and subcortical white matter [38, 39] and is generally thought to represent structural or metabolic dysfunction [40]. The pathogenesis of interictal slow wave remains poorly understood. Keller and colleagues found an increase in neuronal firing during discharge as well as a diminished rate of neuronal activity during the slow wave, corresponding to a period of relative inhibition, suggesting that slow wave represented inhibition of brain function. Additionally, physiologic events that resulted in the interictal discharge were not limited to the seizure focus [41]. There are also perspectives that interictal regional delta slowing represents the epileptogenic process, for its locations are striking in accordance with the epileptic focus [40]. Furthermore, several studies demonstrated a strong relationship between interictal slow wave and diminished rate of neuronal activity in the relevant regions [41, 42]. In this study, we recognized that cerebral autoregulation of patients with interictal slow wave was impaired even and interictal slow wave was an independent predictor of dynamic cerebral autoregulation capability. We speculate that interictal slow wave plays an essential role in the impairment of dynamic cerebral autoregulation through unknown mechanisms and may have an association with postepilepsy stroke. Epidemiologic studies with large sample are needed to confirm this hypothesis.

The study has some limitations. First, the dynamic cerebral autoregulation and EEG were examined separately. Thus, we cannot identify whether the patients had subclinical epileptic discharges during the dynamic cerebral autoregulation measurement, which may influence the cerebral hemodynamics. Second, epilepsy was caused by different reasons, such as juvenile myoclonic epilepsy or mesial temporal lobe sclerosis, which may result in difficulty in explaining the mechanisms of cerebral autoregulation impairment. However, our limitation is not to distinguish the different causes of epilepsy. Furthermore, dementia, a potential factor to influence cerebral autoregulation, is common in patients with epilepsy [43]. Not including the cognitive function test is a limitation of our study.

Our findings raised new questions. It is not easy to determine whether disruption of dynamic cerebral autoregulation during the chronic epileptic phase is just a consequence of epileptic discharges or that it can also contribute to discharges. Further work is required to explore this issue. The cause-and-effect relationship between interictal slow wave and dynamic cerebral autoregulation should also be explored further.

5. Conclusions

The present study documented that dynamic cerebral autoregulation capability is impaired in the patients with epilepsy, especially in those with interictal slow wave. Cerebral autoregulation disruption occurs irresponsive of the discharge location and type, suggesting hemodynamic changes exceeding the epileptogenic focus. Interictal slow wave is an independent factor to predict impaired cerebral autoregulation in patients with epilepsy.

Conflicts of Interest

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

Authors' Contributions

Shan Lv and Zhen-Ni Guo contributed equally to this work.

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Research Article

Comparing Different Recording Lengths of Dynamic Cerebral Autoregulation: 5 versus 10 Minutes

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We compared the dynamic cerebral autoregulation (dCA) indices between 5- and 10-minute data lengths by analyzing 37 patients with ischemic stroke and 51 controls in this study. Correlation coefficient (Mx) and transfer function analysis were applied for dCA analysis. Mx and phase shift in all frequency bands were not significantly different between 5- and 10-minute recordings [mean difference: Mx = 0.02; phase shift of very low frequency (0.02–0.07 Hz) = 0.3°, low frequency (0.07–0.20 Hz) = 0.6°, and high frequency (0.20–0.50 Hz) = 0.1°]. However, the gains in all frequency bands of a 5-minute recording were slightly but significantly higher than those of a 10-minute recording (mean difference of gain: very low frequency = 0.05 cm/s/mmHg, low frequency = 0.11 cm/s/mmHg, and high frequency = 0.14 cm/s/mmHg). The intraclass correlation coefficients between all dCA indices of 5- and 10-minute recordings were favorable, especially in Mx (0.93), phase shift in very low frequency (0.87), and gain in very low frequency (0.94). The areas under the receiver operating characteristic curve for stroke diagnosis between 5- and 10-minute recordings were not different. We concluded that dCA assessed by using a 5-minute recording is not significantly different from that using a 10-minute recording in the clinical application.

1. Introduction

Cerebral autoregulation is a physiological mechanism of maintaining a relatively constant cerebral blood flow (CBF) in response to the systemic hemodynamic change. Dynamic cerebral autoregulation (dCA) can be measured by analyzing the correlation between spontaneous or induced changes in CBF and peripheral blood pressure (BP) [1]. The cerebral blood flow velocity (CBFV) recorded by using a transcranial Doppler ultrasonography (TCD) under normocapnia status is a reliable surrogate of CBF [2].

Continuous CBFV and noninvasive BP recordings are commonly used in the studies of dCA. The dCA under spontaneous CBFV and BP changes can be assessed in time domain (correlation coefficient "Mx" or autoregulatory index "ARI") [3, 4] or frequency domain (transfer function analysis, TFA) [5]. Although there is no gold standard method of dCA assessment, it has been proposed that the minimum data recording length is 5 minutes in order to obtain stable results [6]. In past studies, a common recording length of spontaneous CBFV and BP changes is 5 or 10 minutes [5, 7–15] and a longer recording length of more than 20 minutes was also used [16–18]. In subjects with illness, a long recording time will be vulnerable to motion artifacts due to poor cooperation. Therefore, it is better to have a recording time as short as possible in clinical practices. A study revealed that the ARI, Mx, and phase shift exhibit large variability under a recording time less than 5 minutes [19]. However, whether a longer recording time more than 5 minutes is beneficial in the research or clinical application is unclear.

This study used both Mx and TFA to investigate the agreement between 5 and 10 minutes of recording, as well as comparing their validity for identifying patients with stroke.

2. Methods

2.1. Subjects. This study was approved by the Institutional Review Board of Taipei Medical University, which comprised

the data from our previous study [20] and newly recruited participants. Patients with acute ischemic stroke admitted to the neurology ward of Taipei Medical University Shuang Ho Hospital were consecutively screened for the eligibility of this study. Magnetic resonance imaging and angiography (MRI and MRA), electrocardiography (ECG), extracranial carotid Doppler sonography (ECCD), and transcranial color-coded duplex sonography (TCCS) were the routine exams for each patient with stroke. Patients with atrial fibrillation found in ECG, bilateral poor temporal windows found in TCCS, more than 50% stenosis of internal carotid artery found in ECCD, or more than 50% stenosis of middle cerebral artery (MCA) found in MRA were excluded at initial screening. In the patients who agreed to participate in this study, dCA was measured within 3 months of stroke onset, and stroke severity was measured by using the National Health Institute Stroke Scale (NIHSS) on the day of dCA measurement. Controls without a history of stroke were recruited at the health management center of the same hospital. Written informed consent was obtained from all subjects.

2.2. Dynamic Cerebral Autoregulation Measurement and Analysis. The dCA measurements were recorded when the subjects were supine with head elevated at 30° and normal breathing. The end-tidal CO_2 was measured by using a capnography (Nellcor N85, Medtronic, USA). A TCD monitor (MultiDop-T, DWL, Germany), with 2-MHz probes fixed at temporal region and an insonation depth of 50–60 mm, was used for recording the CBFV in MCA. A finger photoplethysmogram (Finometer Pro, Finapres, the Netherlands), with physiologic calibration ("physiocal") turned on, was used for recording continuous BP. CBFV and BP of 10 minutes were simultaneously sampled at 50 Hz by using a data acquisition device (NI USB-6221 BNC, National Instruments, USA). Recordings were started after 15 minutes of rest and with a stable end-tidal CO_2 level. The signals were synchronized between the TCD monitor and Finapres device [20]. The data were inspected manually before dCA analysis, minor artifacts were removed by linear interpolation, and severe artifacts were excluded from analysis.

The raw waveform was downsampled at 10 Hz without detrending, normalization, or filtering for offline analysis. The dCA was analyzed by using Mx and TFA. The Mx was calculated as the following steps: Pearson correlation coefficients between 20 consecutive 3-second periods (a total of 1 minute) of mean CBFV and BP were calculated, and all correlation coefficients during the recording period were averaged as the Mx [4, 21]. $Mx = 0$ indicates intact dCA, which represents that the changes in CBF were independent of those in BP, whereas $Mx = 1$ indicates absent dCA, which represents that the changes in CBF were totally dependent of those in BP [22]. The TFA was performed by using the algorithm provided by the International Cerebral Autoregulation Research Network (CARNet, http://www.car-net.org/content/resources) with its default TFA parameters. The TFA calculated phase shift, gain, and coherence between the CBFV and BP in very low frequency (VLF, 0.02–0.07 Hz), low frequency (LF, 0.07–0.20 Hz), and high frequency (HF, 0.20–0.50 Hz) bands. By using this TFA algorithm, the default data window length is 102 seconds, and a 5-minute recording comprises 5 windows with 50% data overlap, whereas a 10-minute recording comprises 13 windows with 59.9% data overlap. In the transfer function between CBFV and BP, dCA decreases the influence of BP changes on CBFV. In subjects with intact dCA, the changes in CBFV are smaller and are restored faster than those in BP compared to subjects with impaired dCA [6, 22]. Therefore, a large gain and a small phase shift in TFA represent impaired dCA. In patients with cerebrovascular diseases, Mx was reported larger than controls [18, 23], and phase shifts were reported smaller than controls [23, 24]. In this study, we compared the dCA calculated from the first 5 minutes, the last 5 minutes, and the total 10 minutes to test the stability and agreement of dCA indices between different recording lengths. We furtherly excluded the patients whose VLF phase shift or gain could not be calculated due to unacceptable low coherence (<0.34 in 5 windows, and <0.14 in 13 windows according to the white paper of CARNet [6]). In patients with a substantially low coherence, the TFA results are unreliable due to poor linear correlation between CBFV and BP, and their Mx could be misinterpreted as good dCA [25]. The data of total 88 subjects, including 37 patients with ischemic stroke (age, 56±11 years; 28 males) and 51 controls (age, 47±14 years; 18 males), were enrolled for the final statistical analysis.

2.3. Statistical Analysis. The normality of data was checked by using the Shapiro-Wilk test. The normally distributed data were expressed as mean ± standard deviation (SD), and nonnormally distributed data were expressed as median with interquartile range (IQR). The continuous variables between the patients and controls were compared by using the t-test or the Mann–Whitney U test according to the normality of data. The categorical variables between the patients and controls were compared by using the Fisher's exact test. In the patients with stroke and controls, the dCA of affected side and right side were used for statistical analysis, respectively (the data of controls were from our previous study, in them only the CBFV in right MCA was recorded) [20]. Because most dCA indices were not normally distributed, dCA indices were compared between the first 5-minute, the last 5-minute, and 10-minute recordings by using the Friedman test with post hoc analysis. The agreement and intraindividual correlations between each dCA index from the first 5-minute and 10-minute recordings were tested using the Bland-Altman methods and intraclass correlation coefficient (ICC), respectively. The area under the receiver operating characteristic (ROC) curve of each dCA index was compared between the first 5-minute and 10-minute recordings for identifying patients with stroke in all subjects by using the method proposed by DeLong et al. [26]. $P < 0.05$ was considered statistically significant. Statistical data were analyzed using MedCalc statistical software (version 17.9; MedCalc Software bvba, Ostend, Belgium).

3. Results

The clinical characteristics of the subjects are summarized in Table 1. The age, proportion of male sex, hypertension,

Table 1: Clinical characteristics of the subjects.

	Stroke (+) $n = 37$	Stroke (−) $n = 51$	P value
Age (range)	56 ± 11 (33–80)	47 ± 14 (20–67)	0.001
Sex: male	28 (76%)	18 (35%)	<0.001
Comorbidities			
Hypertension	26 (70%)	13 (25%)	<0.001
Diabetes	14 (38%)	7 (14%)	0.012
Hyperlipidemia	23 (62%)	23 (45%)	0.134
NIHSS (range)	3 ± 3 (0–15)		
Stroke etiology			
LAA	15 (40.5%)		
SVD	22 (59.5%)		

LAA: large artery atherosclerosis; NIHSS: National Institute of Health Stroke Scale, obtained on the day of dCA assessment; and SVD: small vessel disease.

and diabetes in patients with stroke were significantly higher than those in controls. Most patients had mild stroke severity (NIHSS = 3 ± 3). The agreements between dCA assessed for the first 5 minutes, the last 5 minutes, and 10 minutes are presented in Table 2. All 88 subjects had the results of Mx as well as the phase shift and gain in VLF band, but 10 of them did not have the results of phase shift and gain in LF and HF bands due to unacceptable low coherence.

All dCA indices were not significantly different between the first 5 minutes and the last 5 minutes, and the phase shift in all frequency bands and Mx were not significantly different between the 5- and 10-minute recordings. However, the gain and coherence in all frequency bands were significantly higher in each of the first and last 5-minute recording than those in the 10-minute recording. The mean difference of each dCA index between the first 5 minutes and 10 minutes calculated by using Bland-Altman methods agreed with the results of Friedman test. This phenomenon existed in both patients and controls (the results of subgroup analysis are presented in the online Supplementary Table (available here)). The ICC of Mx and all TFA indices between the first 5- and 10- minute recordings were favorable, especially of Mx (0.93), phase shift in VLF (0.87), and gain in VLF (0.94) (Table 2).

The areas under the curve (AUC) of ROC for Mx, phase shift, and gain for identifying patients with stroke in all subjects are presented in Table 3. The validity in identifying patients with stroke was favorable for the Mx (AUC of the first 5- and 10-minute recordings = 0.714 and 0.719, resp.) and phase shift of VLF (AUC of the first 5- and 10-minute recordings = 0.707 and 0.716, resp.). The AUCs for the phase shift in LF or HF and gain in all frequency bands did not significantly differ from random guesses (AUC = 0.5). The AUCs of all dCA indices between the first 5- and 10-minute recordings were not significantly different. Thus, the validity of dCA indices for identifying patients with stroke was not different between the 5- and 10-minute recordings.

4. Discussion

In the current study, all dCA indices remained stable from the first 5 minutes to the last 5 minutes, and the phase shifts in all frequency bands and Mx were not significantly different between the 5- and 10-minute recordings. Moreover, the AUCs of ROC curves for identifying patients with stroke were not significantly different in phase shift in all frequency bands and Mx between the 5- and 10-minute recordings. Therefore, in the study of stroke, the application of dCA based on spontaneous CBFV and BP changes would not be significantly different between the 5- and 10-minute recordings. A study of 16-minute recording revealed that ARI, Mx, and phase shift would be stable after 3, 6, and 5 minutes, respectively [19]. A 5-minute recording length may be sufficient for dCA assessment. However, the gain and coherent were slightly but significantly higher in 5-minute than those in 10-minute recording; the reasons are unclear and need further investigations.

The higher coherence and gain of the 5-minute recording than those of the 10-minute recording might be explained by methodological issues. In this study, we used raw waveform of CBFV and BP for dCA analysis, and the "physiocal" of the Finapres device remained active throughout the recording period. Deegan et al. reported that gain and coherence but not phase shift would decrease as signal artifacts increase when using raw waveform of CBFV and BP for TFA [27], which is similar to our findings. In this study, average of 4 to 6 "physiocal" occurred in a 5-minute recording, and the number of "physiocal" doubled in a 10-minute recording; however, the ratio of artifacts to signals in time is the same between 5- and 10-minute recordings; hence, other mechanisms that decrease gain and coherence may also exist. In Deegan's study of TFA estimated from 1 to 5 minutes of recordings, there was a trend that gain and coherence but not phase shift decreased as the data length increased, and the gain and coherence but not phase shift in a 5-minute recording were significantly smaller than those in a 1-minute recording [27]. In this study, the gain and coherence were not different between the first and last 5 minutes but were smaller in the 10 minutes than in each of the first and last 5 minutes. Therefore, it is possible that gain and coherence decrease as the data length increases which is a nature of TFA rather than a physiological phenomenon. The stability of gain and coherence in TFA has not been tested in large scale or discussed, and it seems that in a 10-minute time scale, gain and coherence may not be stable according to Deegan's and our findings. In previous studies of dCA in stroke, gain has not been reported to differ between patients and controls [12, 14, 21, 28]. If gain is an unstable dCA index, it would be difficult to correlate gain with other physiologic or clinical variables. In the ROC curve analysis in this study, gain was not valid for identifying patients with stroke. However, it is possible that gain would stabilize in a larger time scale for more than 10 minutes, and further investigations are warranted.

This study has limitations. First, we compared the validity only in identifying stroke between 5- and 10- minute recordings, and whether the studies of other diseases could

Table 2: Agreements between dCA assessed for 5 and 10 mins.

All subjects ($n = 88$)	The first 5 mins, median (IQR)	The last 5 mins, median (IQR)	10 mins, median (IQR)	Mean difference ± 95% of agreement between the first 5 mins and 10 mins	Intraclass correlation coefficient between the first 5 mins and 10 mins (95% CI)
Mx	0.37 (0.18–0.48)	0.34 (0.11–0.52)	0.35 (0.15–0.48)	0.02 ± 0.19	0.93 (0.90–0.96)
Phase Shift (Degree)					
VLF (0.02–0.07 Hz)	55 (41–74)	56 (41–73)	57 (45–73)	−0.3 ± 28.1	0.87 (0.81–0.91)
LF† (0.07–0.20 Hz)	39 (29–49)	36 (23–50)	36 (26–47)	0.6 ± 42.0	0.74 (0.62–0.82)
HF† (0.20–0.50 Hz)	9 (−8–19)	9 (−3–22)	6 (−5–17)	−0.1 ± 37.6	0.76 (0.65–0.84)
Gain (cm/s/mmHg)					
VLF (0.02–0.07 Hz)	0.43 (0.32–0.66)	0.47 (0.33–0.69)	0.30 (0.30–0.61)*§	0.05 ± 0.29$^{\#}$	0.94 (0.90–0.96)
LF† (0.07–0.20 Hz)	0.48 (0.34–0.65)	0.47 (0.35–0.62)	0.38 (0.28–0.55)*§	0.11 ± 0.27$^{\#}$	0.77 (0.37–0.89)
HF† (0.20–0.50 Hz)	0.49 (0.37–0.66)	0.48 (0.38–0.65)	0.38 (0.28–0.53)*§	0.14 ± 0.28$^{\#}$	0.78 (0.25–0.91)
Coherence					
VLF (0.02–0.07 Hz)	0.46 (0.29–0.62)	0.46 (0.24–0.63)	0.41 (0.25–0.59)*§	0.04 ± 0.22$^{\#}$	0.80 (0.70–0.87)
LF (0.07–0.20 Hz)	0.33 (0.21–0.46)	0.30 (0.19–0.44)	0.25 (0.15–0.40)*§	0.06 ± 0.14$^{\#}$	0.86 (0.44–0.95)
HF (0.20–0.50 Hz)	0.23 (0.16–0.29)	0.21 (0.16–0.27)	0.15 (0.10–0.21)*§	0.07 ± 0.13$^{\#}$	0.69 (0.11–0.87)

$^{*}P < 0.05$ compared to the first 5 mins; $^{\S}P < 0.05$ compared to the last 5 mins; $^{\#}P < 0.05$ mean difference = 0; $^{\dagger}n = 78$; CI: confidence interval.

Table 3: The accuracy of identifying stroke patients in all subjects in the first 5 mins and 10 mins of recordings.

All subjects ($n = 88$)	Area under the ROC curve (95% confidence interval)	
	5 mins	10 mins
Mx	0.714 (0.607–0.805)*	0.719 (0.613–0.810)*
Phase shift (degree)		
VLF (0.02–0.07 Hz)	0.707 (0.600–0.799)*	0.716 (0.610–0.807)*
LF† (0.07–0.20 Hz)	0.557 (0.439–0.670)	0.568 (0.450–0.681)
HF† (0.20–0.50 Hz)	0.507 (0.391–0.622)	0.510 (0.395–0.625)
Gain (cm/s/mmHg)		
VLF (0.02–0.07 Hz)	0.531 (0.422–0.638)	0.548 (0.438–0.654)
LF† (0.07–0.20 Hz)	0.560 (0.443–0.673)	0.511 (0.395–0.626)
HF† (0.20–0.50 Hz)	0.599 (0.481–0.708)	0.503 (0.388–0.619)

* $P < 0.05$ AUC = 0.5; all areas under the ROC curves were not significantly different between 5 mins and 10 mins; † $n = 78$.

benefit from a longer recording length is unclear. Second, it is unclear whether a recording period longer than 10 minutes would yield a result different from ours. Third, we used raw waveform of CBFV and BP for dCA analysis, and the results of using beat-to-beat data need further investigations.

5. Conclusion

The *Mx* and phase shift assessed under spontaneous CBFV and BP changes are not significantly different between 5- and 10-minute recordings and have the same validity in the study of stroke. However, gain and coherence are higher in the 5-minute recording compared to those in the 10-minute recording. A unified recording length in a single study or between studies could minimize the influences of time-dependent variables.

Conflicts of Interest

All authors report no conflicts of interest.

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Supplementary Materials

Supplementary Table Agreements between dCA assessed for 5 and 10 minutes in (A) patients with stroke and (B) controls. *(Supplementary Materials)*

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