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Review Article

Experimental and Clinical Use of Therapeutic Hypothermia for Ischemic Stroke: Opportunities and Limitations

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Stroke remains a disease with a serious impact on quality of life but few effective treatments exist. There is an urgent need to develop and/or improve neuroprotective strategies to combat this. Many drugs proven to be neuroprotective in experimental models fail to improve patient outcome in a clinical setting. An emerging treatment, therapeutic hypothermia (TH), is a promising neuroprotective therapy in stroke management. Several studies with TH in experimental models and small clinical trials have shown beneficial effects. Despite this, implementation into the clinical setting is still lacking due to methodological considerations as well as hypothermia-related complications. This paper discusses the possible opportunities and limitations of the use of TH in animal models and the translation into the clinic.

1. Introduction

Stroke is the third leading cause of death in industrialized countries, after cardiovascular diseases and cancer, and is the main cause of severe and long-term disability. Moreover, as our society ages, the incidence of stroke will increase and will become a considerable socioeconomic burden on society due to the excessive costs of long hospitalisations, nursing care, and rehabilitation [1–5].

So far, two major strategies in the treatment of thrombotic stroke exist: thrombolysis and neuroprotective therapies. Thrombolysis (or recanalization) with recombinant tissue plasminogen activator (rt-PA) is the only approved and effective therapy after thrombotic stroke thus far. Unfortunately, treatment with rt-PA has important limitations such as a short treatment window (3-4.5 h), reperfusion-associated injury, and hemorrhagic complications. Consequently, less than 10% of all patients with stroke can be treated with thrombolytic agents [1–4, 6–8]. Although other agents including platelet inhibitors (aspirin), anticoagulants (ancrod, heparins), and prourokinase have been used in the management of acute stroke, there is only poor or no evidence that these approaches improve outcome [4, 9, 10]. Alternatively, neuroprotection after an acute ischemic stroke antagonizes, interrupts, reduces, or slows down injurious biochemical and molecular events [11]. Neuroprotective strategies mostly focus on reducing damage in the penumbra and thus improving the outcome after stroke [12]. Some neuroprotective agents have shown promising results in animal studies; however, very few clinical trials show the same neuroprotective effect in patients with an ischemic stroke [13–15].

Therapeutic hypothermia (TH) is one of the best studied neuroprotective therapies. Small decreases in brain temperature are well tolerated and have been shown to confer a significant degree of neuroprotection in several animal models of cerebral ischemia as well as a reduction of the extent of ischemic brain damage and improvement of neurological function [16–21]. Clinical trials and experimental studies in acute stroke indicate that there is an association between body temperature, initial stroke severity, infarct volume, and clinical outcome [22–24]. Increased brain temperature is

associated with deleterious effects on injured brain tissue [24]. Fever, even a mild one, during the first few days of acute stroke is associated with a worse clinical outcome [22, 24, 25]. This is a concern as twenty-five percent of stroke patients have a body temperature of 37.5°C or more within the first 6 hours after the onset of the symptoms [6]. In contrast, clinical observations have revealed that patients whose body temperature was low at admission to hospital have a better neurological outcome than patients with normal or high body temperature [25]. In addition, controlling body temperature below 36.5°C has proven to correlate with good clinical outcome [26]. Therefore, the conclusion can be drawn that increases in brain temperature escalate neuronal damage whereas lowering the brain temperature reduces brain damage [5]. Thus, although a fever is considered to be a normal physiological defensive response to infection, inflammation, and several other conditions, avoiding hyperthermia and controlling the body temperature are necessary to prevent a worse outcome after cerebral ischemia [19, 27, 28].

Understanding the ischemic cascade, including excitotoxicity, free radical production, and inflammatory and apoptotic pathways, is mandatory in order to develop new therapeutic strategies for stroke. Several extensive reviews on the complex ischemic cascade after stroke have been published [2, 3, 25, 29–32]. Briefly, when a brain vessel is occluded, a series of cellular and molecular events occur, starting with a decrease in cerebral blood flow which can interfere with ion homeostasis. Indeed, within minutes to a few hours, the occlusion leads to increased intracellular calcium levels and glutamate release, causing excitotoxicity and a spreading depression throughout the ischemic region. Cells start to depolarize, causing even more calcium influx and glutamate release. Additionally, water will passively follow the ion influx, resulting in cytotoxic edema [1, 31, 33]. After a few hours to a few days, the high intracellular calcium levels lead to an overactivation of several proteolytic enzyme systems that degrade cytoskeletal proteins. Activation of Ca^{2+}-dependent enzymes, including phospholipase A_2, cyclooxygenase, and neuronal nitric oxide synthase results in additional cellular damage and the generation of reactive oxygen species (ROS), which subsequently damage membranes, mitochondria, and DNA. As a result of all these stimulatory influences, an apoptotic and neuroinflammatory response develops [3, 29–31, 34]. Repair and regeneration over the following months will determine the ultimate damage [35]. In all cases, the location of the original occlusion, and whether the occlusion was permanent or transient, is critical in determining the size and severity of the insult [25, 36].

TH protects the brain in various ways. Among these, it retards energy depletion by lowering the metabolic and the enzymatic rate, restores the neurotransmitter balance, reduces the intracellular calcium influx, reduces intracellular acidosis, suppresses the generation of ROS, suppresses infiltration of inflammatory cells, prevents blood-brain barrier disruption, suppresses specific cell death pathways, or upregulates cell survival mechanisms [17, 23, 34, 37, 38]. A review of the mechanisms of action of hypothermia was not in the scope of this manuscript. Therefore, the reader is referred to a number of reviews on this subject [17, 23].

2. Experimental Studies

Several experimental stroke models have been developed over the last decade to mimic human stroke. Of these, occlusion of the middle cerebral artery (MCA) is the closest to the patient's situation. Several strategies have been used to induce focal cerebral ischemia near the MCA including the embolic model, the intraluminal suture MCA occlusion model, the photothrombosis model, and the endothelin-1 model. These models are well described by Durukan and Tatlisumak [3]. The cerebral insult in stroke models may vary from permanent to transient occlusions and transient models are diverse in the occlusion time, varying from 30 minutes to 3 hours [23]. Due to the range of model variables, the severity of functional and structural outcome changes depend on the exact experimental protocol, and thus it is important to emphasise which experimental model is used [39].

In stroke models, different types of animals are used, including rats, mice, rabbits, and gerbils. The rat is most commonly used in experimental studies for several reasons including the resemblance between human and rat cerebrovascular anatomy and physiology, the moderate size of the animal which allows easy monitoring of physiological parameters as well as cytological analysis of the brain, and the relative homogeneity within strains [3]. Mice are preferred when genetic modifications to investigate specific differences in the pathophysiology and ischemic cascade of stroke are required. Transgenic mice offer the advantage of a short gestation time (18.5–21 days) and perhaps most importantly a well-developed set of technologies for introducing genetic modifications [40].

While experimental stroke models are an important tool to identify new treatment strategies, there are a few important pitfalls. There is not "one" ideal ischemic stroke model since human stroke itself is a diverse condition [3]. Each model only mimics some aspects of the pathophysiology and the progression of the human stroke. Furthermore, the species and the strain that are used, even within the same laboratory, may determine the outcome of a treatment strategy. Portelli et al. [41] found that several pharmacological, behavioural, and neurochemical factors were significantly different from one breeding location to another and could even vary in time between rats coming from the same breeding location. So, it is likely that genetic differences have an effect on infarct outcomes in the various stroke models. Ren et al. [42] demonstrated significant differences in temporal window for hypothermic protection among rats from different strains in which MCA occlusion was induced. Furthermore, even if the same model is used, technical manipulations can vary between investigators of different laboratories. The location and the intensity of the lesion as well as its progression can vary accordingly [42].

Currently, experiments are generally performed in young healthy rodents. This is in contrast with the clinical situation, where most patients are elderly and have concomitant pathologies which can affect the outcome of an ischemic incident. Hyperglycemia, for example, plays a role in the severity of stroke and it has been associated with exacerbated

cerebral damage and increased morbidity and mortality in acute ischemic stroke [43, 44]. It is important to take these risk factors and comorbidities into account when testing on animals. Experiments performed in aged rodents suffering from hypertension, hypercholesterolemia, and/or diabetes are therefore more relevant to clinical practice [45, 46]. These conditions were formulated in the original Stroke Therapy Academic Industry Roundtable (STAIR) criteria [47].

Technical complications also compromise the accuracy of animal stroke models. While brain temperature is the critical issue in regard to neuroprotection in stroke, most experimental studies rely on core temperature measurements, which are usually estimated by sampling rectal temperature because it is easy and inexpensive [45]. A few researchers have used telemetry probes to measure temperature, most often in the core but also in the brain [48, 49]. Although some authors claimed that rectal temperature can nicely predict brain temperature, there is conflicting data about this correlation [18, 49]. One study in rodents showed that the core temperature is about 0.7°C higher than brain temperature [49] whereas another study in rodents found that brain temperature was 0.2 to 0.7°C higher than rectal temperature [18]. Since the temperature is not consistent throughout the body or brain, there is no consensus in which brain region measurements should be performed. Not only the brain region is important, but there is also a difference in temperature between the ischemic and the contralateral hemisphere. Even the way TH is induced could even influence the correlation between the core and brain temperature. Measuring the temperature in freely moving animals or animals under anaesthesia may be an important consideration. As minimal changes in brain temperature can have protective effects, standard methods of measuring temperature should be elaborated to allow comparison between different studies.

Knowing the optimal depth of hypothermia is important for clinical studies because of the increasing side effects associated with increased temperature reduction. Depending on the depth of cooling, TH has been classified in different levels: minimal (35°C), mild (32–34°C), moderate (28–32°C), deep (20–28°C), profound (5–20°C), and ultraprofound (<5°C) [17]. From the literature, it is not clear which is the most appropriate target temperature, but mild and moderate hypothermia have been shown to be protective in animal models and cause fewer side effects than deep hypothermia [18, 20, 50]. An animal study comparing mild and moderate TH showed that 33°C was better tolerated than 30°C in a rat model of transient focal cerebral ischemia [18]. Another study showed that treatment with hypothermia of 33-34°C in the reperfusion period of focal cerebral ischemia is superior to all other applied temperatures [51]. A third study showed that postischemic hypothermia of 32°C produced a larger reduction in infarct volume than 27°C [52]. Therefore, it seems that mild TH is superior to moderate TH.

An important observation is that in many animal studies, TH is initiated before or at the onset of the insult. However, this is in contrast with the clinical situation, where most patients reach the hospital several hours after symptom onset. Consequently, neuroprotective agents targeting early events in the ischemic cascade are unrealistic, especially because at that time a significant amount of cerebral tissue is already impaired [1]. Animal research suggests that intraischemic hypothermia is more effective than postischemic hypothermia [23]. However, to better mimic clinical settings, initiation of the TH should be delayed in experimental settings and the optimal time window for inducing hypothermia should be determined. Recently, we showed [53] that in the endothelin-1 rat model, a 2-hour mild hypothermia treatment delayed by 1 hour after the insult is still neuroprotective and improves neurological outcome whereas a delay of 2 hours was ineffective. In other MCA occlusion models, similar results have been reported. For instance, Maier et al. [54] showed that 2 hours of hypothermia reduced the infarct volume, even when delayed up to 90 minutes after a 2-hour MCA occlusion. A study by Ohta et al. [55] showed that the hypothermic treatment could be delayed up to 4 hours after 2 hours MCA occlusion if cooling was prolonged for 48 hours. So it seems that hypothermia is neuroprotective when applied early after the insult and remains beneficial, if the duration of the cooling is prolonged.

Most patients, in clinical trials, receive the hypothermic treatment for several days (1 to 3 days) [56]. However, this is in contrast with most experimental studies, where animals mostly receive short periods of cooling (few hours). A rat study showed that a mild hypothermic treatment (using core temperature telemetry measurements) of 48 hours provided superior protection compared to a hypothermic treatment of 12 hours [48]. Another study showed that 22 hours of mild hypothermia (using rectal temperature measurements) was superior to a 3-hour treatment [57]. These data suggest that short periods of cooling may lead to transient neuroprotection. Therefore, prolonging hypothermia treatment might provide permanent neuroprotective effects. As most patients reach the hospital several hours after the cerebral insult, it is possible that longer treatment durations may be necessary when the initiation of cooling is delayed.

The rewarming phase after hypothermic treatment is also important as rapid rewarming may lead to rebound phenomena and enhanced deleterious ischemic effects. Berger et al. [58] compared fast rewarming (within 20 minutes) with slow rewarming (within 2 hours) and showed that slow rewarming significantly reduced the infarct volume compared to fast rewarming. Therefore, as the rewarming phase plays a crucial role in influencing the outcome after stroke, it is important that the rewarming phase should be studied in experimental studies as well or at least be elaborated upon in the experimental protocol to allow comparison.

Most animals are sacrificed within a few days after ischemic onset preventing the study of long-term events [45, 46, 59]. However, because complex processes of the ischemic cascade can last up to some months after the ischemic insult, it is desirable to monitor the animals for a longer period of time than is currently used.

Currently, most experiments are carried out on male animals only, but researchers should consider the possibility

that the sex and the hormone changes might influence stroke outcome. Suzuki et al. [60] showed that estrogens protect the brain against stroke injury which points to an urgent need for studies comparing males and females, and the hormonal changes with it. Besides sex hormones, poor outcome was also associated with high levels of stress hormones. Studies in rodents showed that stress delays or diminishes recovery of cognitive functions after cerebral ischemia [61].

Another important issue to be addressed is the anaesthetic used in experimental studies. Studies using TH are carried out on anesthetized animals to avoid shivering. However, the choice of the anaesthetic can influence the clinical outcome [62] and therefore, the use of a sham group is mandatory. Furthermore, as the method of cooling depends on the choice of the anaesthetic (such as pharmaceuticals and inhalation anaesthetics), it is important to stress these experimental conditions as well. An important contrast between experimental research and the clinical situation is the use of sedation and ventilation. In the clinical setting, the use of anaesthetics has decreased the last few years because of the potential side effects [22]. While in animal models, most studies are still carried out on anesthetized animals when cooling techniques are used. Animals are mostly cooled via surface cooling techniques, such as with fans, water or alcohol spray, use of a cold room, or administration of some chemicals. Selectively cooling the brain, for example with an extracranial cooling coil, is becoming more popular in experimental rat models [63]. In the clinical situation, however, surface cooling combined with endovascular cooling is commonly used [26, 27, 37, 38, 64, 65].

A selection of outcome parameters should be used when evaluating the neuroprotective effect of TH in animal studies. While histological endpoints are important for demonstrating reductions in infarct volume, these assessments may not necessarily be associated with improved functional and neurological outcome [45]. Several laboratories have consistently shown that TH reduces both the extent of neurological damage and improves neurological function [37]. However, simple measurements of recovery are not sufficient to detect improvements in neurological function, as they do not reflect the complexity of functional impairment and may not accurately predict neuroprotection [45]. Functional outcome should be tested with several sensorimotor behavioural and cognitive tests, such as limb placing, beam walking, grid walking, rotarod, sticky label test, staircase test, and Morris water maze [3, 66]. Subsequently, combined functional and histological endpoints are necessary for examining the protective effect of hypothermia or drugs in stroke models [67].

Physiological parameters are strictly controlled during experimental studies, which is in contrast with the clinical setting. It is possible that neuroprotective agents convey a beneficial effect in animal models because some physiological parameters (such as the body temperature, blood pressure, etc.) are controlled in animal experiments. Clinical evidence suggests that admission of acute stroke patients to a stroke unit with intensive monitoring can lead to improved outcome [68]. Optimal medical care is important to reduce the risk of complications and to stabilize a number of acute physiological parameters that may worsen ischemia, such as high or low blood pressure, fluid and electrolyte management, hypoxemia, body temperature, infections, and blood glucose [12]. A strict control of the physiological parameters in stroke patients should improve the outcome.

In summary, a suitable experimental animal model should meet the following criteria: it should be reliable, minimally invasive, and yield reproducible lesions with minimum variability. Age and comorbidities should be taken into account before conducting an experimental study. The physiological variables should also be monitored and maintained within normal ranges [3]. Not only the acute effects of TH, but also the long-term effects should be studied. The model has to be relevant to human stroke and consider all the above-mentioned impediments when these experiments are translated into clinical settings. Although further research is needed to investigate the optimal conditions for TH, the current knowledge suggests that TH should be initiated as soon as possible, and if the treatment is delayed longer, cooling should be considered. Several studies also suggest that mild hypothermia is neuroprotective and produces fewer complications than deep cooling. As the rewarming phase strongly influences the outcome after stroke, it is crucial that all studies use slow rewarming phases.

3. Clinical Studies

There is an urgent need to improve the translation of preclinical research into the clinic. To help the translation of animal studies to human clinical trials, the STAIR criteria were set up to improve the understanding of experimental data and extrapolation to a clinical setting. The STAIR provides recommendations for clinical development of acute stroke therapies, including elimination randomization bias, calculation of sample size, specification of the inclusion and exclusion criteria, blind assessment of the outcome, use of multiple species, and reporting animals excluded from the analysis [1, 47].

As the ischemic cascade is so complex, it is possible that different types of stroke (ischemic or hemorrhagic) benefit from different therapeutic protocols [1]. When running a clinical trial, patients with the same stroke severity should be enrolled, in order to produce comparable results. Of these, patients who have had an earlier stroke or other comorbidity, should be placed in a subgroup during the analysis as it might influence the outcome [11]. Before including patients in a clinical trial, inclusion and exclusion criteria should be well defined and optimized. The protocol has to include cutoff points for the National Institute of Health Stroke Scale (NIHSS) and the Barthel index and should have clear outcome measurements. As hypothermia is less effective in ischemia without reperfusion, any neuroprotective strategy should, if possible, be combined with recanalization therapy.

A number of small clinical trials of hypothermia have already been performed in stroke patients (Table 1), such as the "Copenhagen Stroke Study" [70], the "Cooling for Acute Ischemic Brain Damage" (COOL AID) study [73], and the "Intravascular Cooling in the Treatment of Stroke-Longer

Table 1: Clinical studies with therapeutic hypothermia in acute stroke.

First author, year [ref.]	Number of patients	Sedated or awake (+ antishivering drugs)	Method of cooling	Time to treatment (h)[1]	tPA[2]	Target temperature (°C) + place of measurement	Duration of cooling	Rewarming rate	Outcome
Schwab et al., 1998 [69]	25	Sedated with fentanyl and propofol	Cooling blanket, cold infusion, cold washing	4–24 Mean 14	−	33 Bladder	48–72 h	Passive rewarming in 24 h	SSS[4] and BI[5] (4 weeks and 3 months)
Kammersgaard et al. 2001, [70]	17	Awake (pethidine)	Cooling blanket, cold air	<12 Mean 3	−	35.5 Tympanic and rectal	6 h	In 4 h to 36.5°C	SSS[4] (6 months)
Schwab et al., 2001 [71]	50	Sedated with midazolam or propofol and morphine or fentanyl	Cooling blanket, alcohol, ice bags	4–75 Mean 22	−	33 Bladder	24–72 h	Passive rewarming in 11–24 h	NIHSS[6] (4 weeks) mRS[7] (3 months) BI[5] (3 months)
Georgiadis et al., 2001 [72]	6	Sedated with midazolam andfentanyl	Endovascular cooling	12–58 Mean 28	−	33 Bladder	48–72 h	1°C/8 h Max 0.2°C/h	NA[3]
Krieger et al., 2001 [56]	10	Sedated with propofol	Cooling blanket, ice water	Mean 6	+	32 Bladder	12–72 h	0.21°C/h	mRS[7] (3 months)
De Georgia et al., 2004 [73]	18	Awake (meperidine and buspirone)	Endovascular cooling, warming blanket	<12 Mean 9	−	33 Esophageal	24 h	0.2°C/h	NIHSS[6] and mRS[7] (30 +7 days)
Guluma et al., 2006 [22]	10	Awake (meperidine and buspirone)	Endovascular cooling, warming blanket	<6	+	33 Blood	24 h	0.3°C/h 12 h	NA[3]
Kollmar et al., 2009 [74]	10	Awake (pethidine and buspirone)	Ice-cold saline, warming blanket	<3 Mean 2	+	35.5 Tympanic	NA[3]	NA[3]	NIHSS[6] (1 day and at discharge (± 4.5 days))
Hemmen et al., 2010 [75]	58	Awake (meperidine and buspirone)	Endovascular cooling, warming blanket	0–3 3–6	+	33 Blood	24 h	0.3°C/h 12 h	NIHSS[6] (1, 30 and 90 days) mRS[7] (90 days)

[1]Time from the insult to initiation of treatment; [2]tPA: tissue plasminogen activator (+: administered; −: not administered); [3]NA: not available; [4]SSS: Scandinavian Stroke Scale; [5]BI: Barthel Index; [6]NIHSS: National Institutes of Health Stroke Scale; [7]mRS: modified Rankin scale.

tPA Window" (ICTuS-L) study [75]. The "Copenhagen Stroke Study" showed that modest hypothermia (35.5°C) by surface cooling can be achieved in conscious patients following an acute stroke [70]. The COOL AID study showed that endovascular cooling (33°C) is feasible in conscious patients with moderate to severe ischemic stroke [73]. The ICTuS-L study showed that endovascular cooling (33°C) can be combined with standard thrombolytic therapy [75]. However, further research with larger patient groups is necessary to evaluate the efficacy of hypothermia after ischemic stroke as recent studies were not able to show reduced mortality after an insult [22, 56, 69–75].

Early initiation of hypothermia after stroke gives the best outcomes, but if hypothermia is delayed, longer cooling times may also be protective [38, 76] and thus, as most stroke patients reach the hospital several hours after the insult, many trials use long therapeutic windows compared to animal studies (see Table 1). Although a long cooling time seems attractive, it may increase the risk of complications [17, 37, 76]. Prolonged hypothermia not only increases the risk for side effects, it also has an influence on every organ system [59].

Although several small clinical trials show that TH could be safe for stroke patients, there are still a number of complications that need to be overcome [22, 56, 69]. The most common complications related to hypothermia are pneumonia, hypovolemia, arrhythmias, hyperglycemia, bradycardia, thrombocytopenia, hypertension, hypotension, increased intracranial pressure, electrolyte abnormalities like hypokalemia, and metabolic acidosis [16, 27]. There are also complications related to fast rewarming, such as intracranial hypertension [27].

As mentioned above, besides the control of the body temperature, it is also important to control other physiological parameters such as blood glucose, blood pressure, fluid and electrolyte management, hypoxemia, cardiac arrhythmias, and infections [12]. Clinical evidence suggests that admission of acute stroke patients to a stroke unit with intensive monitoring can improve the outcome [68]. Most studies agree that it is difficult to keep all of the metabolic and physiologic variables that might influence the outcome constant across different centers [27]. However, this should be their goal in order to reduce the risk of a bad outcome and the risk of complications in stroke patients. Blood markers, such as NSE and S100β, should also be included, as they can give an indication of the prognosis and the outcome after stroke.

TH seems a very promising treatment for stroke if investigators can reduce the complications related to the treatment, but there are also other limitations. Endovascular cooling compared to surface cooling may be better to control the body temperature more precisely but it is more invasive [22]. Patients treated with TH have to be admitted to an intensive care unit, this may generate practical and logistical problems [46].

Finally, the lack of translation of experimental studies into human clinical trials reflects the lack of resources as well as shortage of sponsoring from companies and governments [46, 67]. There is an urgent need for larger clinical trials with a sufficient number of patients, which might reveal small but significant differences in outcome and low-incidence complications [22]. Despite all limitations, researchers still believe that TH could become the standard therapy in stroke management, as it already showed promising results in cardiac arrest patients [22, 50, 77].

4. Conclusion

As only a small percentage of stroke patients can be treated with thrombolytic agents, there is an urgent need to develop and to improve neuroprotection strategies. TH seems to be the most promising neuroprotective therapy in experimental models with encouraging results in the clinical setting. The current knowledge from both experimental and clinical research suggests that TH should be initiated as soon as possible after stroke onset in order to achieve the best outcome. Prolonged cooling should be considered if the initiation of the hypothermia treatment is delayed. As the rewarming phase strongly influences the outcome after stroke, it is crucial that all studies use slow rewarming phases. Currently, it can be considered that mild hypothermia is neuroprotective and produces less complications than deep cooling. At the same time, controlling physiological parameters and the temperature during the hypothermic treatment are essential for successful clinical or experimental trials.

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