

Hyperthermia exacerbates ischaemic brain injury

C. X. Wang^{1,2*}, A. Stroink¹, J. M. Casto², and K. Kattner^{1,2}

Hyperthermia frequently occurs in stroke patients. Hyperthermia negatively correlates with clinical outcome and adversely affects treatment regimens otherwise successful under normothermic conditions. Preclinical studies also demonstrate that hyperthermia converts salvageable penumbra to ischaemic infarct. The present article reviews the knowledge accumulated from both clinical and preclinical studies about hyperthermia and ischaemic brain injury, examines current treatment strategies and discusses future research directions.

Key words: cerebral, hyperthermia, infarction, stroke, temperature

Introduction

Stroke is a disorder affecting approximately 15 million people worldwide. It is the third leading cause of death in the majority of industrialised countries and also in many developing countries (1–3). More than 85% of strokes are due to ischaemic brain injury; haemorrhagic brain injury accounts for the remaining cases (1). Although thrombolysis with tissue plasminogen activator (tPA) is the only effective pharmacological treatment proven in randomised-controlled multicentre clinical trials, more than 95% of patients are ineligible to receive this treatment. Unfortunately, neuroprotective therapies have not proven to be effective treatment strategies. To date, 15 000 patients have participated in more than 65 clinical trials of neuroprotective agents (4, 5). Despite enormous efforts, all compounds reaching clinical trials have failed due to a lack of demonstrable efficacy or problems with intolerable adverse effects.

Correspondence: Chen Xu Wang*, 232A FSA, Illinois State University, Normal, IL 61790-4120, USA. Tel: +1 309 438 4494; Fax: +1 309 438 3722; e-mail: cwang@ilstu.edu

¹The Central Illinois Neuroscience Foundation, Bloomington, IL, USA

²Department of Biological Science, Illinois State University, Normal, IL, USA

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Hyperthermia is a common complication of ischaemic stroke; studies show it is detrimental to the ischaemic injured brain and correlates with poor outcome (6). Furthermore, hyperthermia also adversely affects treatment regimens that work under normothermic conditions. For example, previous animal studies have shown that mild hyperthermia abolishes the effects of an otherwise neuroprotective *N*-methyl-d-aspartate (NMDA) agonist, MK-801 (7). Similarly, in a recent study, we demonstrated that hyperthermia masks the beneficial effects of tPA in a focal embolic model of cerebral ischaemia, despite the enhanced fibrinolytic activities of tPA due to increased temperature (8).

While there is extensive research on the effects of *hypothermia* on neuronal damage (beyond the scope of this discussion) and putative pharmacotherapy, less attention has focused on molecular mechanisms of *hyperthermia* in ischaemic brain injury. The present article reviews the current knowledge about hyperthermia and ischaemic brain injury, accumulated from both clinical and preclinical studies, with intentions of stimulating more research in both clinical and preclinical levels.

Body temperature and thermoregulation

Body temperature is regulated by the hypothalamus, which requires integrated autonomic, endocrine and skeletomotor responses. Although both anterior and posterior hypothalamic areas are involved in temperature regulation, the detectors of body temperature, both low and high temperatures, are located in the anterior hypothalamus. The anterior hypothalamus contains cold-sensitive and warm-sensitive neurons which serve as the thermoregulatory centre. The activity of these neurons is highly influenced by the temperature of local blood flow. Warm-sensitive neurons increase firing when local tissue is warmed; likewise, cold-sensitive neurons actively respond to local cooling. The warm-sensitive neurons, in addition to responding to local warming, are generally excited by warming of the skin or spinal cord and are inhibited by cooling of skin or spinal cord. The cold-sensitive neurons exhibit opposite behaviors. Thus, these neurons serve to integrate thermal information from the periphery with that

from the brain and act to maintain the normal range of body temperature (9).

Hyperthermia refers to a pathological increase in body temperature when the thermostatic centre in the hypothalamus is unable to compensate for temperature alteration (10–12). Hyperthermia occurs when the body produces more heat than it can dissipate, and it has been observed in both stroke patients and animals with experimentally induced ischaemia. Several mechanisms may contribute to increasing body temperature. Hyperthermia can result from damage to the thermostatic centre when infarction is concentrated in the hypothalamus. Support for this hypothesis is evidenced in preclinical studies demonstrating that spontaneous increases in body temperature occur in experimental animals following cerebral ischaemia (13). Peripheral infection can also cause hyperthermia, especially when the patient is comatose (14–16).

Clinical studies

Correlation between hyperthermia and stroke outcome

The relationship between body temperature on admission and stroke severity was investigated in a prospective study of 390 patients with acute stroke (17) (Table 1). Within 6 h of

stroke insult, body temperature independently relates to initial stroke severity, infarct size, mortality and poor outcome. For each 1°C increase in body temperature the odds of poor outcome rise by 2.2. To examine whether this relationship was also valid in patients admitted at later time points, the researchers extended the analyses to patients admitted between 6 and 24 h after stroke onset (18). Body temperature was found to independently and significantly related to stroke severity and mortality in patients admitted between 6 and 12 h, but not in patients admitted between 12 and 24 h from stroke onset. Mortality is higher at 60-month poststroke in patients presenting with hyperthermia in the initial phase of onset (<6 h), suggesting that hyperthermia may have long-term effects (19). That hyperthermia correlates with poor stroke outcome is also observed by other groups (13, 20, 21). Findings from several retrospective studies also support that hyperthermia correlates with higher mortality, more severe neurological deficits and less favourable final outcomes (15, 16, 22, 23).

In a study of 725 patients, Boysen and Christensen (12) examined the relationship between body temperature and outcome, at various time points, in patients with both ischaemic and haemorrhagic stroke. No correlation was found in patients admitted within 2 h of stroke onset and outcome at 3 months. For patients admitted within 6 h of stroke onset, lower body temperature was associated with less favourable

Table 1 Hyperthermia and ischaemic stroke in clinical studies

Type	Patient (number)	BT recorded (h post ictus)	Hyperthermia (%)*	Major findings	References
<i>(A) Prospective</i>					
	390	6	25	Hyperthermia worsens mortality and outcome. BT correlates with initial stroke severity, lesion size, mortality and outcome in survivors. For 1°C increase in BT, the relative risk of poor outcome rises by 2.2	(17, 19)
	398	12 and 24	N/G	BT correlates to stroke severity and mortality in patients admitted between 6–12 h from stroke onset, but not admitted 12–24 h	(18)
	260	72	60.8	Hyperthermia initiated in 24 h from insult associated with poor outcome and large infarcts. The earlier the hyperthermia occurs, the higher relation between the BT increase and brain damage	(14)
	725	24	N/G	At 10–12 h after stroke onset, increased BT relates to stroke severity and poor outcome. Initial increased BT (<8 h) does not relate to stroke severity and outcome	(12) [†]
	183	168	43	Higher BT correlates with poor stroke outcome	(20) [†]
	229	24	37.6	Hyperthermia correlates with higher infarct volume, poor outcome	(21)
<i>(B) Retrospective</i>					
	346	72	N/G	Higher BT correlates with larger infarct volume and more severe neurological deficits	(22)
	100	24	53	Hyperthermia associated with unfavourable outcome following thrombolysis with tPA	(24)
	150	72	31	Hyperthermia correlated to mortality	(15)
	3790	N/G	N/G	Hyperthermia correlated to morbidity and mortality	(25)
	509	N/G	N/G	Hyperthermia associated with both short- and long-term mortality. 1°C increase in BT increases relative risk of 1-year mortality by 3.4	(23) [†]
	119	48	32	Increased BT associated with more severe neurological deficits	(16)

*Hyperthermia occurred in the recording period. [†]Includes ischaemic and haemorrhagic stroke. N/G, data not given; BT, body temperature.

outcome. However, starting at 8 h and for every 2-h interval over the next 10 h, higher body temperatures correlated with poor outcomes.

Because clinical studies with small numbers of patients often provide conflicting results, Hajat *et al.* (25), conducted a retrospective meta-analysis using published data. Analyses from the data of 3790 patients revealed that hyperthermia is highly associated with both morbidity ($P < 0.0001$) and mortality ($P < 0.00000001$). These data clearly demonstrate that hyperthermia is harmful to the injured brain.

Hyperthermia and thrombolysis

In 100 consecutive patients treated with tPA for acute stroke, patients with unfavourable outcomes had elevated body temperatures when compared to patients with favourable responses to thrombolytic therapy (24). After adjustment for baseline characteristics, the presence of hyperthermia was significantly associated with a reduced probability of better outcome following thrombolytic treatment.

Hyperthermia and infection

Hyperthermia with or without an infectious cause was also studied in stroke patients (14–16). In a study of 158 stroke patients with hyperthermia within first 72 h, an infectious cause was found in 91 patients (57.6%). Bronchopulmonary infection was identified in 47 patients, urinary infection in 40 patients and thrombophlebitis in nine patients. Body temperature and mortality rate are higher in patients with hyperthermia of infectious aetiology than those with hyperthermia of noninfectious origin in all time periods studied (up to 72 h after stroke onset). Hyperthermia commencing within the first 24 h from stroke onset independently relates to larger infarct volumes, increased neurological deficits and functional dependency at 3 months.

In summary, convincing evidence supports that hyperthermia correlates with increased mortality, severe neurological deficits and poor outcome. While randomised-controlled clinical studies are needed to verify whether hypothermia can reduce ischaemic damage, currently, the main efforts should be directed towards an immediate and effective reduction of body temperature when it is higher than 37.5°C, particularly in the early phase of stroke as high temperature in this period independently contributes to poor prognosis. Further, studies demonstrate that infection, particularly pulmonary and urinary, is a frequent complication and a major reason for increased body temperature in acute stroke patients. Clinicians should always search for an infectious origin in acute stroke patients and any infection should be controlled properly and effectively.

Preclinical studies

Focal ischaemia

Elevation of body temperature aggravates ischaemic brain injury

The deleterious effects of hyperthermia in ischaemic brain injury are more clearly demonstrated in animal models (Table 2). In a permanent model of focal cerebral ischaemia the effects of hyperthermia on ischaemic injury were examined. Chen *et al.* (26) reported that when the brain temperature was increased to 40°C either 1 h before or immediately after ischaemic insult and maintained for a period of 1 h after the injury, infarct volume, measured 4 days after the injury, was significantly larger in the animals receiving hyperthermia treatment than in normothermic rats.

The effects of increased brain temperature were also compared between transient and permanent models of ischaemic injury (28). In the transient model, inducing 39°C hyperthermia for 2 h, starting immediately after middle cerebral artery (MCA) occlusion, significantly increased infarct volume in the cortex after a survival period of 3 days. This treatment does not result in significant changes to infarct volume in the permanent model. Further analyses show that in the transient model, the infarct volume positively correlates to cerebral blood flow during the first 30 min of recirculation. Brain temperature also positively correlates to cerebral blood flow during MCA occlusion and the early recirculation period. These results suggest the net effect of hyperthermia is harmful to the injured brain even with improved local blood flow.

To investigate whether delayed hyperthermia has detrimental effects on the pathological outcome of focal ischaemia, brain temperature was increased to 39–40°C at 24 h after MCA occlusion. Infarct volumes were measured on day 4 after MCA occlusion. Delayed hyperthermia extended ischaemic injury from the caudoputamen to the overlying cortex in most animals with brain temperatures of 40°C. The cortical infarct volume increased by 6.6-fold and total infarct volume by threefold following hyperthermia treatment. In addition, this treatment also worsened neurological deficits. However, treatment with 39°C did not exacerbate the brain damage, indicating both the timing and degree of temperature modulation are important factors in influencing the thermal effects on ischaemic outcome (27).

Recently, we have also studied hyperthermia and ischaemic brain injury using a focal embolic model of stroke in rats (8, 27). In these studies, body temperature was elevated before and continued for 3 h after the ischaemic induction. Hyperthermia (39°C) worsened neurological deficits, and significantly increased infarct volume, measured at 48 h after the MCA occlusion. In addition, the mortality rate was also significantly higher in the hyperthermic rats than in normothermic rats. Findings from a second study showed

Table 2 Hyperthermia and ischaemic stroke in preclinical studies

Model	Species	Hyperthermia (°C)	Initiate (Duration) (h post ischaemia)	Major findings	References
<i>(A) Focal</i>					
AI. Brain temperature					
	Rat	39–40	24 (3)	Hyperthermia 40°C increases infarct volume by 3 fold at 4 days post-MCA occlusion, but not 39°C	(27)
	Rat	40	1 prior or 0 (1–2)*	Hyperthermia, starting at both 1 h before or 1 h after ischaemia, increases infarct volume	(26)
	Rat	39	0 (2)	Hyperthermia increases infarct volume in transient MCA occlusion model, but not in permanent MCA occlusion model	(28)
	Rat	39	0 (2-5)	Hyperthermia increases extracellular glutamate release	(29)
AII. Rectal temperature					
	Rat	39	0-5 (2)	Hyperthermia enhances tPA-induced recanalisation and increases infarct volume	(30)
	Rat	N/A	N/A	Spontaneous hyperthermia occurs with hypothalamic injury. Spontaneous hyperthermia (4–8 h after ischaemia) does not increase infarct volume	(31)
	Rat	N/A	N/A	Spontaneous hyperthermia increases infarct volume, antagonises beneficial effects of reperfusion and accelerates penumbra to infarct	(32)
	Rat	N/A	N/A	Spontaneous hyperthermia nullifies the neuroprotective actions of NMDA antagonist	(7)
	Rat	38–39	0-3 prior (3)	Hyperthermia increases infarct volume, worsens neurological deficits, antagonises actions of thrombolytic agent	(8, 33)
	Rat	N/A	N/A	Selective hypothalamic infarction produces significant and sustained spontaneous hyperthermia	(13)
<i>(B) Global</i>					
BI. Brain muscle temperature					
	Gerbil	39	0 prior (5 min)	Hyperthermia exacerbates ischaemia-induced cell death, causes complete destruction of neurons in CA1	(34)
BII. Temporalis muscle temperature					
	Dog	38–39	0-3 prior (1)	Small increase of temperature worsens neurological function and severity of injury	(35)
	Rat	39	0 (0-3)	Hyperthermia enhances ischaemic brain damage and mortality, fosters transformation of ischaemic injury into infarction, accelerates morphological appearance of ischaemic brain injury	(36)
BIII. Skull temperature					
	Rat	39	Prior (5, 10, or 15 min)	Increase of temperature by 2°C enhances brain damage	(37)
BIV. Rectal temperature					
	Rat	N/A	N/A	Spontaneous hyperthermia (>38.5°C) occurs from 21 to 63 h following ischaemia. Depressing body temperature to normothermic diminishes neuronal damage	(38)
	Rat	39.6	24 (3)	Delayed hyperthermia exacerbates ischaemic neuronal injury	(39)

*Before ischaemic induction.

that hyperthermia (39°C) exacerbated brain injury which is consistent with the first study. Furthermore, the second study also showed that treatment with hyperthermia (38°C) increased infarct volume, ischaemic brain oedema and seizure activities compared with normothermic rats.

Spontaneous hyperthermia

Spontaneous hyperthermia has been examined in several studies using focal models of ischaemic brain injury. Reglodi *et al.* (32) reported that hyperthermia occurs in rats experiencing transient ischaemia with durations of 90 min, 2 h and permanent ischaemia. Temperature starts to increase at

15–20 min after MCA occlusion and reaches 39–40.5°C during the first hour. Sustained hyperthermia is observed during the rest of the first 24 h. Spontaneous hyperthermia has also been observed by several other groups (31, 40).

Spontaneous hyperthermia increases infarct volume in both permanent and transient MCA occlusion (32), which is due to accelerated transformation of salvageable tissues to infarct. The peripheral zone of a focally ischaemic region, the so-called ischaemic penumbra, is known to be a temperature-sensitive area. In 2,3,5-triphenyltetrazolium chloride-stained brain sections, the border zone between normal (red) and infarct (white) zones are separated by tissue with pink colour. These

pink tissues are an early indicator of ischaemia and are also believed to represent the penumbra region. Compared with the normothermic controls, the infarct size increases and penumbra area decreases significantly at 4, 12 and 48 h in the hyperthermic animals, indicating the transformation of penumbra to infarct.

To examine whether the hypothalamus, the thermoregulating centre, is responsible for spontaneous hyperthermia, temperature variations were recorded when ischaemic damage was induced only in hypothalamus or in both neocortex (MCA territory) and nonneocortical structures (13). Rats with hypothalamic infarction exhibit persistent hyperthermia for 72 h, whereas rats with infarction at both neocortex and nonneocortical structures display transient hyperthermia. In addition, a lesion involving the medial hypothalamus alone, by occlusion of tuberal artery, causes hyperthermia irrespective of the obstruction of the anterior cerebral artery or anterior choroidal artery. These results provide evidence that hypothalamus is involved in the occurrence of hyperthermia.

Hyperthermia affects the efficacy of neuroprotective and thrombolytic agents

The noncompetitive NMDA antagonist dizocilpine maleate (MK-801) is an effective treatment to reduce infarction in animal models of focal ischaemia. Unexpectedly, it was ineffective in a transient model, occluding the MCA with an intra-arterial suture (7). In exploring the possible reasons for this difference among models, researchers found that spontaneous hyperthermia occurred following MCA occlusion. To examine whether hyperthermia nullified the protective effects of MK-801, ischaemic injured rats were divided into groups in which body temperature was allowed to increase spontaneously (39–39.5°C) and groups in which body temperature was maintained within normal range during ischaemia. MK-801 failed to reduce infarct size in animals with spontaneous hyperthermia. In contrast, this drug treatment markedly reduced infarct volume in animals when the body temperature was controlled. These results indicate that amelioration of focal ischaemic damage cannot be expected if hyperthermia is allowed to occur.

Hyperthermia also blunts the beneficial action of reperfusion in ischaemic brain injury (32). In a suture model of ischaemic brain injury, reperfusion is achieved by removing the intra-arterial nylon suture. A comparison between animals with different reperfusion times revealed that infarct sizes were smaller when reperfusion occurs at 90 min than 120 min, even in animals with postischaemic spontaneous hyperthermia. However, infarct sizes of the hyperthermic rats with 120-min transient ischaemia exceed those of the normothermic rats with permanent ischaemia, indicating hyperthermia abolishes the beneficial actions of reperfusion.

In a focal embolic model of ischaemic brain injury a positive relationship between body temperature during the initial phase of infarct development and final infarct volume has

been demonstrated (30). Despite better re-canalisation of the occluded arteries, treatment with tPA enlarged the size of infarcted tissue in the hyperthermic animals. These results suggest that hyperthermia can convert an effective treatment to harmful toxic agent in the injured brain. Based on these results, authors of this study have suggested that hyperthermia should be avoided in clinical trials of thrombolytic therapy and in patients with acute ischaemic stroke in general. This study is highly significant since thrombolytic therapy with tPA is presently the only proven effective pharmacological treatment for acute stroke patients.

We also studied whether hyperthermia eliminates the beneficial effects of tPA treatment in an embolic model of cerebral ischaemia (8). We found that hyperthermia significantly increased infarct volume and brain oedema. Treatment with tPA significantly reduced infarct volume in normothermic and 38°C rats. However, this treatment did not reduce the infarct volume when the body temperature was increased to 39°C. Treatment with tPA reduced brain oedema only in the 38°C group but not in the 39°C group. Neurological deficit scores were significantly higher in the hyperthermic rats than in the normothermic rats. Treatment with tPA improved neurological deficits in the 38°C group but not in the 39°C group. In addition, *in vitro* experiments showed that hyperthermia increased the fibrinolytic activity of tPA. Thus, these data clearly show that hyperthermia abolishes the therapeutic actions of thrombolytic treatment with tPA, even though the fibrinolytic activity of tPA is increased with increasing temperature. These findings indicate that the increased fibrinolytic activity of tPA is offset by its deleterious actions; and the net effect of hyperthermia in ischaemic brain injury is neurodestructive.

Global ischaemia

The findings from global model of ischaemic brain injury are consistent with those from focal models. In a global model of cerebral ischaemia induced by vessel occlusion combined with systemic hypotension in rats, increased brain temperature accelerated ischaemic injured cells to necrotic death (36, 37). Mortality rates were also increased in animals with increased brain temperature (36). Subsequent study reveals that transient hyperthermia, even imposed 1 day following a brief episode of global ischaemic insult, exacerbates the extent of ischaemic neuronal injury (39). Conversely, prevention of transient postischaemic hyperthermia reduces the extent of neuronal damage (37). In gerbils, hyperthermia results in accentuated ischaemia-induced loss of CA1 neurons in hippocampus. Increasing cerebral temperature to 39°C completely destroys the neurons in CA1 regions and also produces an extension of ischaemic neuropathology to regions CA2, CA3 and CA4 where there is no significant loss of neurons in normothermic animals (34).

Occurrence of spontaneous hyperthermia is also observed in the global model of ischaemia (38). In rats subjected to 10 min of transient ischaemia induced by occlusion of common carotid arteries in combination with hypotension, body temperature persistently increased to above 38.5°C starting at 1 day from reperfusion and continuing for 3 days. Depressing body temperature to normothermic range by the antipyretic agent, dipyrone, markedly diminished neuronal damage in the neocortex and hippocampus when evaluated 7 days after ischaemia. Cooling the animals to normothermic levels with physical therapy was also beneficial for the injured brain. These observations suggest that the processes leading to neuronal death are very sensitive to temperature increase, not only during the ischaemic insults, but also in the late reperfusion phase, which is relevant to the design of clinical therapeutic treatment as stroke patients usually arrive at the hospital a few hours following the insult.

Hyperthermia affecting functional changes and histological changes has also been studied in a global model of cerebral ischaemia using large animals (35). Complete cerebral ischaemia, 12.5 min in duration, is produced by arterial hypotension plus intracranial hypertension. Compared with the controls (37°C), dogs with increased brain temperature of 1 or 2°C exhibit significantly worse neurologic function during a 72-h postischaemic observation period. At the end of the experiments, 72 h after ischaemia or at the time of ischaemia-related death, the severity of injury was determined histologically. Structures rich in grey matter were more susceptible to ischaemic injury and more likely to be influenced by temperature increases. When compared with the 37°C controls, significantly increased injury was observed in both 38°C and 39°C dogs. The severity of injury was also higher in the 39°C dogs than in the 38°C dogs. Moreover, mortality rates also increased in dogs with increased body temperature.

In summary, compelling evidence from both focal and global models of ischaemic cerebral injury demonstrates that the brain is very sensitive to temperature changes. Increased brain temperature, either spontaneous or extrinsically induced, worsens neuronal function, accelerates injury processes, converts salvageable tissue to macro-infarct and aggravates stroke outcome. If these observations are transferable to stroke patients, these data strongly suggest hyperthermia is harmful to the injured brain and hyperthermia should be corrected efficiently and effectively.

Possible mechanisms of increased cerebral damage by hyperthermia

Currently, there is no comprehensive explanation for why hyperthermia exacerbates damage to neuronal cells following ischaemic brain injury, but it is likely due to multiple mechanisms working in combination (Table 3, Fig. 1). These mechanisms include metabolic changes, free radical production, increased inflammatory reactions and extracellular excitatory

neurotransmitters, damage of the microvessels and cytoskeletal structures and activation of death signals.

Metabolic functions

In a model of global cerebral ischaemia in cats, the effects of mild whole body hyperthermia were investigated on metabolic recovery during recirculation using *in vivo* phosphorus-31 nuclear magnetic resonance spectroscopy (45). Hyperthermia (40.6°C) was induced 1 h before ischaemia and maintained during 1.5–2 h of recirculation. In hyperthermic cats, β -adenosine 5'-triphosphate (β -ATP) and phosphocreatine (PCr) as well as the ratio of PCr to inorganic phosphate fail to return to preischaemic levels during recirculation, in contrast to normothermic cats. Hyperthermia also caused lower intracellular cerebral pH during recirculation than in the normothermic cats. These data suggest mild hyperthermia, concurrent with global cerebral ischaemia in cats, worsens metabolic and physiological functions.

Following reductions in cerebral blood flow and oxygen supply, high-energy phosphorylated compounds, such as ATP and PCr, are reduced and tissue acidosis occurs. On the other hand, hyperthermia, if present, increases energy utilisation. Studies using newborn swine have shown a linear relationship between brain energy utilisation and brain temperature over a range of 28–41°C. An elevation in brain temperature increases energy utilisation by 5.3% per 1°C (48). Thus, increased energy utilisation further depletes energy substrates and cultivates accumulation of ions and toxic metabolites, which can increase the brain's vulnerability to injury under ischaemic conditions. Furthermore, ischaemia denatures proteins and leads to protein aggregation within neurons (49). Renaturing of proteins by a cellular chaperone system requires ATP. Therefore, protein denaturation may cause damaging effects to the injured brain when energy stress is combined.

Free radicals

Hyperthermia enhances ischaemia-induced increases of free radicals. In a model of global ischaemic brain injury, the levels of hydroxyl radicals were examined using a method based on chemical trapping in the form of stable adducts 2,3- and 2,5-dihydroxybenzoic acid following salicylate administration (42, 43). In normothermic rats, the levels of hydroxyl radicals decreased during ischaemic injury and significantly increased during reperfusion. Hyperthermia treatment results in even higher increases of hydroxyl radicals. In addition, results also show that these hydroxyl radicals arise within brain parenchyma itself.

Free radicals are extremely active compounds that can interact with lipids, enzymes and DNA to produce a variety of harmful actions. The brain is particularly vulnerable to oxidative damage because it is rich in unsaturated fatty acids and relatively poor in antioxidant defenses. The injurious role

Table 3 Possible mechanisms of hyperthermia worsening stroke outcome

Model	Species	Hyperthermia (°C)	Initiate (Duration) (h post ischaemia)	Major findings	References
<i>(I) Preclinical studies</i>					
A. Focal					
	Rat	39	0 (1)	Hyperthermia increases degradation of cytoskeletal proteins by enhancing calpain activities	(41)
	Rat	38–39	0.3 prior (3)	Hyperthermia compromises microcirculation, damages BBB	(8)
B. Global					
	Rat	39	3 min prior (7 min)	Hyperthermia increases production of free radicals	(42, 43)
	Rat	39	5 min (15 min)	Hyperthermia enhances the reduction of PKC activities	(44)
	Cat	40.6	1 prior (1.5–2)	Hyperthermia results in lower intracellular pH, and β -ATP phosphocreatine	(45)
	Gerbil	39	Prior (5 min)	Raising intracerebral temperature increases the inhibition of CaM kinase II, and aggravates the loss of MAP = 2	(34)
	Rat	39	0 (0.3)	Hyperthermia increases permeability of microvessels	(46)
<i>(II) Clinical studies</i>					
				Hyperthermia correlates with higher plasmatic pro-inflammatory cytokines	(21)
				Hyperthermia increases glutamate and glycine in the CSF in stroke patients	(47)

CSF, cerebrospinal fluid; MAP, microtubule-associated protein.

of these radicals in the setting of ischaemia is evident from studies demonstrating that free radical scavengers confer protection from ischaemic damage (5).

Inflammatory reactions

A clinical study involving 214 stroke patients was carried out to determine the relationship between pro-inflammatory cytokines and hyperthermia as it relates to larger infarct size (21). Cytokines were detected at admission and also 48 h later. Results from this study show that the plasmatic levels of interleukin (IL)-6 and tumour necrosis factor α (TNF α), measured at admission, significantly correlate to infarct volume. The plasmatic levels of IL-6, TNF α , intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule-1, measured at 48 h after the admission, also correlate to infarct volume. Moreover, a significant association was found between the cytokines (IL-6, TNF α and ICAM-1) and poor stroke outcome.

Preclinical studies have also shown that inflammatory reactions occur in the ischaemic injured brain (50). These reactions are characterised by a sequential series of processes, including the release of pro-inflammatory cytokines, increased expression of endothelial adhesion molecules and chemotactic factors, activation of microglia and macrophages, and leucocyte infiltration. Overwhelming evidence supports that inflammatory reactions contribute considerably to the pathogenesis of ischaemic brain injury through the increase of secondary expansion of ischaemic infarction. In addition, pro-inflammatory cytokines, TNF α , IL-6 and IL-1 β , which are pivotal for the inflammatory response, are known to be

pyrogenic. Thus, hyperthermia may be part of a stroke-induced inflammatory reaction (11).

Excitatory neurotransmitters

Hyperthermia exacerbates ischaemic brain injury by modifying the release of excitatory neurotransmitters. Clinical study reveals that the concentrations of glutamate and glycine in the cerebrospinal fluid increase significantly in patients with hyperthermia (47). Higher body temperature also relates to worsening neurological deficits and larger infarct volume. In preclinical research, the hyperthermia-enhanced release of glutamate has been investigated in the ischaemic injured brain by measuring extracellular concentration of glutamate using intra-cortical microdialysis (29). In hyperthermic rats (39°C), peak glutamate concentration in the cortex during MCA occlusion is 31-fold above baseline, compared with 6.5-fold elevations in normothermic rats. Significantly increased glutamate release is also observed in the brain following global ischaemia (6).

Glutamate is the major endogenous excitatory neurotransmitter in the central nervous system (51). Increased release of these neurotransmitters causes neurotoxicity through an excessive activation of pro-synaptic glutamate receptors, which leads to an increase in intracellular free calcium ion concentration, triggering a cascade of events which eventuate in neuronal death.

Microcirculatory and blood–brain barrier (BBB)

The permeability of the BBB has been examined in the global model of cerebral ischaemia, by evaluating the leakage of

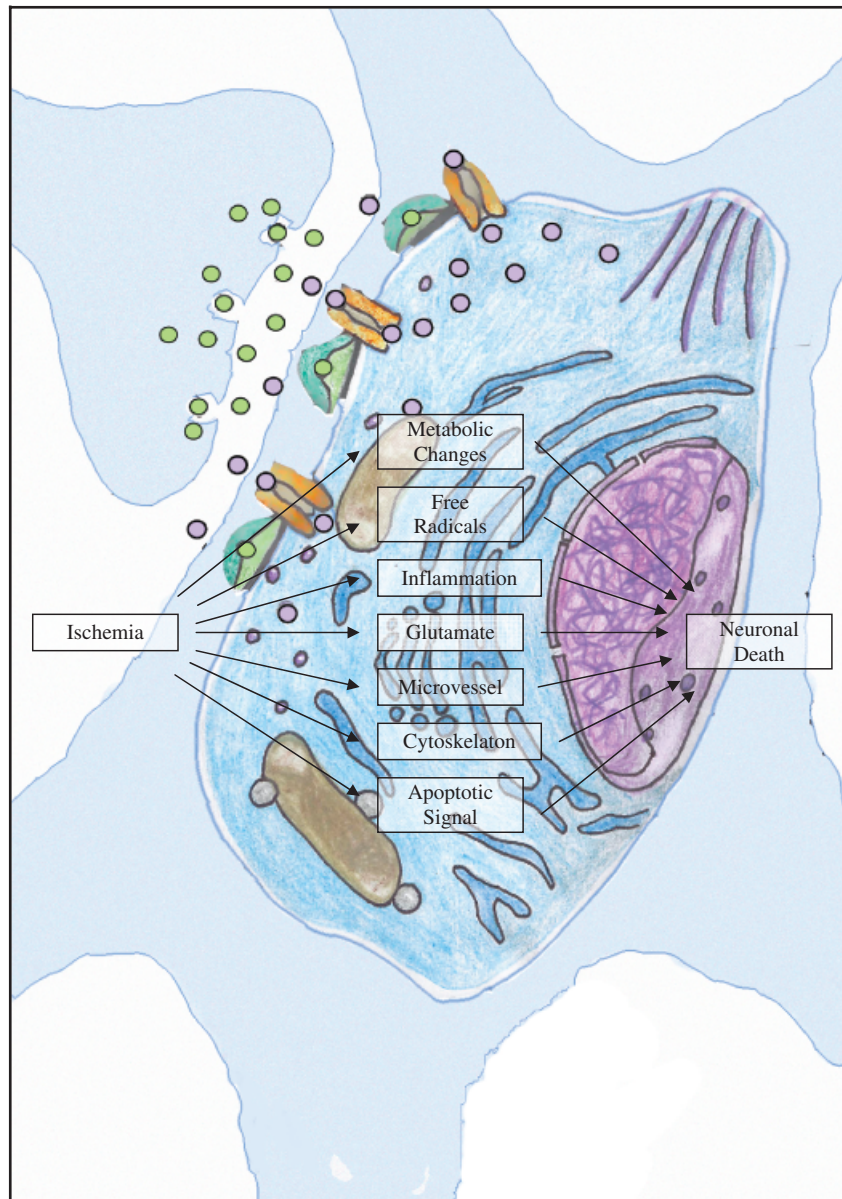


Fig. 1 Mechanisms of hyperthermia-enhanced neuronal death in the ischaemic injured brain.

horseradish peroxidase (46). Hyperthermia (39°C), instituted during the 20-min ischaemic induction, significantly increased the BBB breach in the CA1 area of the hippocampus and ventrolateral thalamus. Importantly, the sites of increased vascular permeability were spatially correlated with dark shrunken (damaged) neurons, indicating hyperthermia may enhance neuronal death through BBB damage.

We have examined whether hyperthermia affects the microcirculation, and BBB permeability, in an embolic model of focal ischaemia (8, 52). In these studies, perfusion deficits were revealed using Evan blue staining and BBB permeability was evaluated by detecting extravagated Evan blue dye. Compared with normothermic rats, perfusion deficits in the hyperthermic rats (39°C) were significantly larger at 3 and 6 h after MCA

occlusion, indicating that hyperthermia compromises the microcirculation in the ischaemic injured brain. To examine the effects of hyperthermia on BBB damage, concentrations of extravagated Evan blue dye were measured in the striatum, the cortex above the striatum and the cortex more peripheral to the striatum. The extravasations of Evan blue dye were significantly increased in both the peripheral cortex and in the striatum in the rats with hyperthermia (39°C).

Cytoskeleton damage

Spectrin is a cytoskeleton protein; it is degraded by activated calpain, a calcium-sensitive cysteine protease. Immunohistochemical localisations of spectrin breakdown products have

been studied using an MCA occlusion model (41). In normothermic rats spectrin immunoreactivity is present occasionally in the cortical neurons at 1 h; sparse spectrin immunoreactivity appears at 4 h after reperfusion. No spectrin immunoreactivity could be detected at 24 h after reperfusion. In contrast, moderate to intensive spectrin immunostaining is present in the cortical neurons at 1 h after reperfusion in hyperthermic animals. At 4 and 24 h, most brains exhibit dense immunoreactivity associated with morphologically shrunken neurons. These data support that hyperthermia is involved in the damage of cytoskeleton during ischaemic brain injury.

Microtubule-associated protein (MAP)-2, a cytoskeleton-related protein, selectively associates with neuronal soma and dendrites. Microtubule-associated protein-2 regulates the stability of microtubules and also mediates the interaction of cytoskeletons and other cell components. Changes of MAP-2 have been evaluated with immunohistochemistry using a global model of ischaemia in gerbils (53). Compared with sham-operated animals, MAP-2 immunostaining significantly decreased at 48 h following ischaemic injury. However, compared with the normothermic animals, MAP-2 immunostaining was significantly lower in the hyperthermic animals at 24 h, but not at 48 h, after ischaemia, indicating that hyperthermia accelerates enzyme activities responsible for MAP-2 degradation in the injured brain. These results suggest that hyperthermia may foster the disassembly of microtubules and destruction of cytoskeleton structures, which in turn contributes to neuronal death.

Kinase activities and other signal transduction proteins

The effects of hyperthermia on the activities of two kinases, CaM kinase II and protein kinase C (PKC), have been studied in the ischaemic injured brain. CaM kinase II activity is significantly inhibited in the brain following ischaemia (34). Hyperthermia intensifies ischaemia-induced inhibition of CaM kinase II activity. CaM kinase II plays an important role in the transient increase of intracellular Ca^{2+} . This kinase is predominantly expressed in neurons, and comprises 2% of total hippocampal proteins. The alteration in CaM kinase II caused by ischaemia may trigger changes in calcium-regulated processes, such as ion fluxes, transmitter release and cell transport mechanisms which eventually lead to neuronal death.

In the vulnerable brain regions, ischaemia induces significant reductions in PKC activities, which are irreversible for up to 24 h after reperfusion (44). Similar to the changes in CaM kinase II, hyperthermia also leads to further reduction of PKC activities. Upon activation, PKC enzymes are translocated to the plasma membrane. The activated PKC can phosphorylate many types of proteins, such as cytoskeleton protein, and thus alter their functions. Therefore, hyperthermia-induced alteration of PKC activities may contribute to the secondary injury processes in the brain following ischaemia.

Summary and future directions

Findings of clinical studies support that hyperthermia relates to poor stroke outcome and increased mortality. Preclinical studies manipulating brain temperature have provided more direct and clear evidence that hyperthermia is detrimental to the ischaemic injured brain. These findings have prompted investigators to plan clinical trials on cooling therapy with physical or pharmacological treatments in acute stroke patients with the aim of improving stroke outcomes.

Acetaminophen is a safe, clinically proven antipyretic that acts at the hypothalamic thermal regulating centre. In a randomised-controlled trial, early administration of acetaminophen, 3.9 g/day, to afebrile acute stroke patients resulted in very modest, but not significant, reduction in body temperature (54). However, treatment with a daily dose of 6 g of acetaminophen significantly lowers body temperature by 0.4°C in acute stroke patients (55). Further studies are needed to determine whether this small reduction in body temperature leads to improved outcome.

Owing to its anti-platelet properties, aspirin is recommended treatment in ischaemic stroke as soon as a CT or MRI scan has ruled out intracerebral haemorrhage (11). Preclinical studies also show that aspirin prevents glutamate-induced neurotoxicity, reduces inflammatory reactions, improves microcirculation and protects the ischaemic injured brain (56, 57). These data support aspirin as an ideal antipyretic medication in every case of hyperthermia, if the cause of ischaemic stroke is not from a cardio-embolic origin and no other general contraindications exist. However, more research is needed to examine whether treatment with aspirin is effective in stroke patients with hyperthermia.

Physical cooling therapy has also been evaluated to reduce body temperature. Normal range of body temperature (36–37°C) can be attained in hyperthermic stroke patients within 3–3 h by application of a water-perfused cooling mattress (58). An even lower body temperature can be achieved with more intensive physical cooling therapy. However, physical cooling is limited by its potential adverse effects including cardiac arrhythmia, metabolic derangements and propensity for infection. Additionally, physical cooling also requires sedatives and muscle relaxation adjuvants.

Currently, no evidence exists from randomised trials to support the routine use of pharmacological or physical cooling in acute stroke. Given the high incidence of hyperthermia in stroke patients, and compelling evidence suggesting that hyperthermia has deleterious effects in stroke outcome, multi-centre randomised-controlled clinical trials are worthwhile to examine whether the prevention of hyperthermia by prophylactic therapy can lead to an improvement in stroke outcome.

Although the present article intends to review the research progress during recent decade, two pioneer studies on hyperthermia and stroke are worth mentioning. The first retrospective study was reported by Hindfelt in 1976 (59). In this study, prognostic influence of hyperthermia was analysed in

110 patients with varying neurological disabilities. Results from this study support that hyperthermia correlates to an unfavourable prognosis with respect to residual symptoms. Based on this observation, the author suggests that hyperthermia, irrespective of its genesis, should be intensely combated during early stages of ischaemic stroke. In 1994, Castillo et al. (60) reported the first prospective clinical trial. In this study, 177 patients with ischaemic stroke are analysed. Results demonstrated that hyperthermia is associated with poor outcomes in patients with ischaemic stroke. Both temperature and glucose levels were significantly higher in the patients that died than in the surviving patients during a 6-month follow-up period; however, further analyses indicate that temperature is the only variable that has a significant influence on morbidity.

More than 30 years have passed since the first retrospective study on hyperthermia and ischaemic stroke was reported. Only recently has attention been focused on the actions of hyperthermia, in comparison to the large amount of research that has been conducted on hypothermia. Additionally, little attention has been devoted to research towards understanding the cellular and molecular mechanisms of hyperthermia-enhanced injury processes. Although research has shown that hyperthermia affects changes in some biological markers, it is not clear if these changes play significant roles in neuronal death or if they are simply the epiphenomena of cell injury. Further studies to identify these cellular and molecular mechanisms will provide important information not only for mechanisms of hyperthermia-enhanced neuronal death but also for the fundamental mechanisms of ischaemic injury. This knowledge will certainly aid in the design of neuroprotective strategies for the treatment of stroke patients.

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