Neuroprotective effect of preoperatively induced mild hypothermia as determined by biomarkers and histopathological estimation in a rat subdural hematoma decompression model

Laboratory investigation

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Object. In patients who have sustained a traumatic brain injury (TBI), hypothermia therapy has not shown efficacy in multicenter clinical trials. Armed with the post hoc data from the latest clinical trial (National Acute Brain Injury Study: Hypothermia II), the authors hypothesized that hypothermia may be beneficial in an acute subdural hematoma (SDH) rat model by blunting the effects of ischemia/reperfusion injury. The major aim of this study was to test the efficacy of temperature management in reducing brain damage after acute SDH.

Methods. The rats were induced with acute SDH and placed into 1 of 4 groups: 1) normothermia group (37°C); 2) early hypothermia group, head and body temperature reduced to 33°C 30 minutes prior to craniotomy; 3) late hypothermia group, temperature lowered to 33°C 30 minutes after decompression; and 4) sham group, no acute SDH (only craniotomy with normothermia). To assess for neuronal and glial cell damage, the authors analyzed microdialysate concentrations of GFAP and ubiquitin carboxyl-terminal hydrolase-L1 (UCH-L1) by using a 100-kD probe. Fluoro-Jade B–positive neurons and injury volume with 2,3,5-triphenyltetrazolium chloride staining were also measured.

Results. In the early phase of reperfusion (30 minutes, 2.5 hours after decompression), extracellular UCH-L1 in the early hypothermia group was significantly lower than in the normothermia group (early, 4.9 ± 1.0 ng/dl; late, 35.2 ± 12.1 ng/dl; normothermia, 50.20 ± 28.3 ng/dl; sham, 3.1 ± 1.3 ng/dl; early vs normothermia, p < 0.01; sham vs normothermia, p < 0.01, analyzed using ANOVA followed by a post hoc Bonferroni test). In the late phase of reperfusion (> 2.5 hours after decompression), extracellular GFAP in the early hypothermia group was also lower than in the normothermia and late hypothermia groups (early, 5.5 ± 2.9 ng/dl; late, 7.4 ± 3.4 ng/dl; normothermia, 15.3 ± 8.4 ng/dl; sham, 3.3 ± 1.0 ng/dl; normothermia vs sham; p < 0.01). The number of Fluoro-Jade B–positive cells in the early hypothermia group was significantly smaller than that in the normothermia group (normothermia vs early: 774,588 ± 162,173 vs 180,903 ± 42,212, p < 0.05). Also, the injury area and volume were smaller in the early hypothermia group in which hypothermia was induced before craniotomy and cerebral reperfusion (early, 115.2 ± 15.4 mm³; late, 344.7 ± 29.1 mm³; normothermia, 311.2 ± 79.2 mm³; p < 0.05).

Conclusions. The data suggest that early, preoperatively induced hypothermia could mediate the reduction of neuronal and glial damage in the reperfusion phase of ischemia/reperfusion brain injury.

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Key Words • ischemia/reperfusion injury • hypothermia • rat • traumatic brain injury • microdialysis • biomarker • neuronal degeneration

Abbreviations used in this paper: LPR = lactate/pyruvate ratio; MABP = mean arterial blood pressure; NABISH II = National Acute Brain Injury Study: Hypothermia II; SDH = subdural hematoma; TBI = traumatic brain injury; TTC = 2,3,5-triphenyltetrazolium chloride; UCH-L1 = ubiquitin carboxyl-terminal hydrolase-L1.
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ological and behavioral consequences of TBI using many experimental models and different delivery paradigms. Unfortunately, however, therapeutic hypothermia has not shown overall efficacy in multicenter trials, probably due, at least in part, to the heterogeneous nature of the brain damage mechanisms in patients who have sustained TBI. In a recent large clinical trial on severe TBI, post hoc subgroup analysis demonstrated the possibility that early induced hypothermia might have a specific beneficial effect in patients with acute SDHs. This subgroup of patients with acute SDH demonstrates the worst outcome of any category of severe TBI, and 60% will die or remain severely disabled.

Based on these data from the study by Clifton et al., we hypothesized that hypothermia may be beneficial for acute intracranial hematomas via blunting the effects of ischemia/reperfusion injury. To date, no well-controlled preclinical studies have been undertaken to test the efficacy of early cooling in an acute hematoma model of severe TBI. We therefore used the well-characterized acute SDH model in rats to evaluate the efficacy of temperature management in reducing brain damage after acute SDH.

We measured the effect of early and delayed moderate hypothermia (33°C) by using cell counts (Fluoro-Jade staining, Millipore) of neurons dying due to activation of both necrotic and apoptotic cell death pathways and by an independent volumetric estimation of the amount of brain damage, using the mitochondria TTC staining method. Since there is no effective method to predict outcome in patients with acute SDH, we also tested the ability of a new neural biomarker, UCH-L1, and a glial marker, GFAP, to determine the amount of cell death and the effect of the hypothermic therapy in this model.

The prognostic utility of brain biomarkers, such as S100β protein or neuron-specific enolase, has been studied extensively in patients with severe TBI. Serum sampling of these biomarkers may be unable to differentiate brain injury from multiple trauma, including skeletal injuries. Additional influences, such as BBB dysfunction or renal dysfunction, make using serum concentrations alone problematic for S100β measurement.

For a more direct estimation of brain damage, the microdialysis technique can be used. Microdialysis has demonstrated promise in neuromonitoring of severely brain-injured patients. Extracellular biomarkers, such as glucose, lactate, pyruvate, glyceral, and glutamate, have been measured with 20-kD cutoff microdialysis probes in the clinical setting. The LPR has been said to be effective in the detection of ischemic events. The LPR, however, is influenced by the primary injury and delayed events, and it is often difficult to correlate LPR values with cellular/neuronal survival and therefore patient outcome. For a more direct understanding of neuronal and glial viability, we measured 2 recently discovered biomarkers using 100-kD cutoff microdialysis probes in an acute SDH model in rats. Glial fibrillary acidic protein and UCH-L1 were studied as markers of glial and neuronal cell damage, respectively. Glial fibrillary acidic protein is said to be one of the more clinically reliable serum biomarkers for head injury. Furthermore, the molecular weight of GFAP is 50 kD, and this allows a high recovery rate with the 100-kD microdialysis technique. UCH-L1 has also been said to be a sensitive and specific neuronal biomarker, able to predict injury severity and death after severe TBI in humans. UCH-L1 is a compact cytosolic protein with a low molecular weight (~ 24 kD), allowing measurement of UCH-L1 concentrations in extracellular fluid, relatively easily with 100-kD microdialysis techniques. In the past there have been no studies, laboratory or clinical, that correlate microdialysate levels of UCH-L1 and GFAP with their predictive ability for outcome. Our secondary aim was therefore to analyze these biomarkers in serum and extracellular fluid (measured using microdialysis), comparing their levels before and after craniotomy and establishing their value as markers for monitoring injury and therapeutic response in this SDH brain injury model.

Methods

Animal Groups

Adult male Sprague-Dawley rats (weight 300–350 g, Harlan Laboratories) were randomly divided into 4 treatment groups (10 rats in each group). The rats underwent SDH induction, brain microdialysis catheter placement, tail artery cannulation, and temperature manipulation as described below (Fig. 1). In the normothermia group, the head temperature was maintained at normothermic levels (37°C) during the course of the experiment. The early hypothermia group underwent hypothermia induction (33°C) 30 minutes prior to decompressive craniotomy and removal of the hematoma to mimic a clinical situation in which hypothermia induction could be started as soon as acute SDH was diagnosed by CT scanning and while the operating room was being prepared. Hypothermic treatment was continued 3 hours after decompression. The late hypothermia group received hypothermia induction (33°C) 30 minutes after decompression surgery, and it was maintained for 3 hours. The sham group did not receive induced SDH but underwent craniotomy. The head temperature of these rats was maintained at normothermic levels (37°C) during the course of the experiment.

Surgical Procedure

The animals were maintained on a 12-hour/12-hour light/dark cycle and given food ad libitum. All animal procedures followed guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Miami’s Institutional Animal Care and Use Committee.

All animals were anesthetized initially with 3% isoflurane, 70% N2O, and a balance of 30% O2 delivered in a Perspex chamber, with subsequent endotracheal intubation and mechanical respiration as previously described. The tail artery was cannulated with a polyethylene catheter for blood pressure monitoring, blood sampling, and obtaining the autologous blood needed for acute SDH induction. Blood gas analysis was performed 4 times throughout the procedure (Fig. 1 and Table 1) to...
control ventilation of the animal. A PaO₂ of approximately 100–150 mm Hg and a PaCO₂ of 30–40 mm Hg were aimed for to mimic clinical conditions.

**Temperature Manipulations**

The head and rectal temperatures were maintained at 33°C in the early and late hypothermia groups by a combination of a cooling/heating system (Medi-Therm III, Gaymar Industries, Inc.) and a local cooling fan/heating lamp. In the normothermia and sham-treated groups, head and rectal temperatures were maintained at 37°C during the course of the experiment. The head temperature was measured using a thermistor probe placed in the right temporalis muscle and was estimated as the brain temperature.²⁹

**Subdural Hematoma Induction**

Details of the method used to produce SDH are described in previous reports by our group.¹⁸,³⁸,³⁹ A midline scalp incision was made, and a 3-mm-diameter bur hole was drilled 2 mm to the left of the sagittal suture and 3 mm behind the coronal suture (Fig. 2). With the aid of an operating microscope, the dura mater was incised, and a blunt-tipped, J-shaped, 23-gauge needle was inserted into the subdural space. Quick-setting cyanoacrylate glue was used to set the needle and seal the bur hole. The hematoma was then induced by injecting 350 μl nonheparinized autologous blood into the subdural space over a period of 7 minutes, allowing it to clot in situ. After injection, the induction needle was cut off and sealed. In the sham-treated group, the needle was set in place but no blood was injected.

Two and a half hours after induction of the SDH, a craniotomy measuring 15 × 6 mm was made using a saline-cooled dental drill (Fig. 2). The hematoma was then removed using saline irrigation and forceps after widely opening the dura mater. Hemostasis of superficial blood vessels was achieved using bipolar diathermy if needed. The scalp was closed over the craniotomy without replacing the bone to mimic the clinical practice of decompressive craniotomy.

**Extracellular Biomarker Measurement With Microdialysis**

For this measurement (7 rats in each group), we used a CMA 12 microdialysis probe (CMA Microdialysis AB), which had an active membrane length of 4 mm and a molecular weight cutoff at 100 kD. One hour before acute SDH induction, a second bur hole was drilled 2 mm to the left of the sagittal suture and 2 mm behind the lambdoid suture for microdialysis probe insertion (Fig. 2).
The probe was inserted into this bur hole at an angle of 10° from the horizontal plane and at a depth of 6 mm, as previously described. This insertion technique made for optimal placement of the dialyzing membrane within the cerebral subcortical “penumbra” area. Probes were precalibrated in vitro to ensure that interprobe variation was minimal. The dialysis probes were continuously perfused with physiological saline with 4% bovine serum albumin added at 0.3 μl/min. Microdialysis sampling was delayed by 1 hour after insertion into allow the brain to adapt to the presence of the probe. Dialysate sampling began to be collected from 2.5 hours before craniotomy and were sampled 4 times, that is, the ischemic phase (0–2 hours after acute SDH induction), the craniotomy phase (0.5 hours before and after craniotomy), the early reperfusion phase (0.5–2.5 hours after craniotomy), and the late reperfusion phase (2.5–3.5 hours after craniotomy) (Fig. 1). Frozen microdialysate vials were later analyzed for biomarkers. Also, at 2 similar time points in the ischemic and reperfusion phase, 0.6 ml of blood was collected (Fig. 1) and centrifuged at 2500 rpm for 10 minutes. From these centrifuged blood samples, serum was saved and stored at −80°C for biomarker analyses, in parallel with the microdialysate samples.

Quantitative detection of UCH-L1 in serum and microdialysate was performed using a proprietary southwestern chemistry–based enzyme-linked immunosorbent assay (Banyan Biomarkers, Inc.) and recombinant UCH-L1 as standard. For quantification of GFAP in serum and microdialysate, a novel rat enzyme-linked immunosorbent assay (Banyan Biomarkers, Inc.) was used.

Degenerative Neuron Counting With Fluoro-Jade B Staining

Twenty-four hours after the surgery, 7 animals in each group were anesthetized (3% isoflurane, 70% N₂O, and 30% O₂ for 5 minutes) and were perfused transcardi-
Fig. 2. Schematic representation of craniotomy and bur hole placement. The area of craniotomy extended from the lambdoid suture to 15 mm anterior to the lambdoid. The medial and lateral borders were the sagittal suture and superior temporal line, respectively. This made the width approximately 8 mm. The first bur hole, for hematoma induction, was 3 mm in diameter and was placed 2 mm to the left of the sagittal suture and 3 mm behind the coronal suture. The second bur hole, which allowed for insertion of the microdialysis probe, was 3 mm in diameter and was placed 2 mm to the left of the sagittal suture and 2 mm posterior to the lambdoid suture. The probe was inserted toward the front of the head, at a 10° angle from the horizontal and at a 6-mm depth from the brain surface.

ally with isotonic saline for 2 minutes (80 ml), and then with 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4 (360 ml). The brains were embedded in paraffin and serial sectioned (20 μm thick). One in 5 of the serial sections was stained with Fluoro-Jade B. The sections were air dried at 50°C, deparaffinized by incubation in 100% ethanol for 3 minutes and 70% ethanol for 1 minute, and washed with distilled water. The sections were then incubated for 10 minutes in 0.06% potassium permanganate followed by 30 minutes in Fluoro-Jade B solution. The sections were dried on a slide warmer, cleared with xylene, and coverslipped using Cytoseal (Richard-Allan Scientific).

To determine neuronal degeneration in bilateral cortex and hippocampus, serial sections (16 μm thick after shrinkage, 360 μm apart) from −2.2 to −4.0 mm to bregma were quantified in an unbiased, systematic manner by a blinded observer using the physical fractionator method following the workflow in StereoInvestigator 7.50.1 software (MicroBrightField, Inc.) with an Axioskop microscope (Carl Zeiss MicroImaging, Inc.). In each rat, the cortex area to be counted is defined as the subcortical parietal area between the midline and the rhinal fissure. The hippocampus and subcortex were contoured at 5 μm ventral to the hilus of the dentate gyrus. The volume of the hippocampus and subcortex was calculated using image-analyzing software (lenaraf220, Vector Japan Co.). The total volume of injury was calculated by multiplying the area in each section by the distance between the sections (2 mm).

**Measurement of Injury Volume With TTC Staining**

We quantified the ischemic area and volume of injury by TTC staining at 24 hours after the craniotomy in each of the 4 treatment groups (7 rats in each group). The brain was cut coronally every 2 mm using a Lucite brain matrix (Ted Pella, Inc.), and each section was stained with 2% of TTC for 30 minutes. The injured area was defined as an unstained, white area in each section. For every section, a high-resolution photograph was taken using a digital camera (DSC-T70, Sony), and the volume of injury was calculated using image-analyzing software (lenaraf220, Vector Japan Co.). The total volume of injury was calculated by multiplying the area in each section by the distance between the sections (2 mm).

**Statistical Analysis**

All data are expressed as the mean ± SEM. Fluoro-Jade B–positive cell counts and injury volumes were analyzed with 1-way ANOVA, followed by a post hoc Bonferroni test. Physiological data and the ischemic area were compared using 2-way ANOVA, followed by a post hoc Bonferroni test. Differences were considered significant at p < 0.05. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc.).

**Results**

**Physiological Parameters**

Physiological parameters of MABP, arterial blood pH, PaO₂, and PaCO₂ are given in Table 1. Over the course of the experiments, all physiological values were within the normal range, and there were no significant differences between the various experimental groups in terms of MABP, pH, PaO₂, and PaCO₂. As expected, head (brain) temperatures were significantly lower in the early hypothermia group than in the other groups at the time of decompressive craniotomy and 30 minutes after craniotomy (p < 0.001) and in the early hypothermia and the late hypothermia groups compared with the sham and normothermia groups at 1, 1.5, 2.5, and 3.5 hours after craniotomy (Fig. 3).

**Degenerative Neuron Counting**

The number of Fluoro-Jade B–positive cells was predominantly greater in the ipsilateral and subcortical regions under acute SDH (Fig. 4G). Compared with the normothermia group (E-Hypo), mild hypothermia (33°C) was induced 30 minutes before craniotomy. In the late hypothermia group (L-Hypo), cooling began 30 minutes after craniotomy. At the time of decompressive craniotomy, head temperature was significantly different between early and late hypothermia groups. Values are expressed as the mean ± SEM. Normo = normothermia group.
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In the early hypothermia group, the number of Fluoro-Jade B–positive cells in the early hypothermia group was significantly less, especially in the ipsilateral subcortical region (normothermia vs early: 774,588 ± 162,173 vs 180,903 ± 42,212, p < 0.05) (Fig. 4G).

Injury Area and Volume Measurement With TTC Staining

In the early hypothermia group, the injury area was smaller than in the late hypothermia and normothermia groups in each section (Fig. 5A and B). Moreover, the total injury volume in the early hypothermia group was significantly smaller than in the other treatment groups (115.2 ± 15.4 mm³ in the early hypothermia group, 344.7 ± 29.1 mm³ in the late hypothermia group, and 311.2 ± 79.2 mm³ in the normothermia group; p < 0.05) (Fig. 5C).

Concentrations of Biomarkers in Serum and Cerebral Microdialysate

The UCH-L1 microdialysate concentration in the early induced hypothermia group was lower than that in the normothermia and late hypothermia groups in the early phase of reperfusion (30 minutes, 2.5 hours after decompression; early, 4.9 ± 1.0 ng/dl; late, 35.2 ± 12.1 ng/dl; normothermia, 50.20 ± 28.3 ng/dl; sham, 3.1 ± 1.3 ng/dl; early vs normothermia, p < 0.01; sham vs normothermia, p < 0.01) (Fig. 6A). Also, in the late phase of reperfusion (> 2.5 hours after decompression), extracellular GFAP in the early hypothermia group was lower than that in the normothermia and late hypothermia groups (early, 5.5 ± 2.9 ng/dl; late, 7.4 ± 3.4 ng/dl; normothermia, 15.3 ± 8.4 ng/dl; sham, 3.3 ± 1.0 ng/dl; normothermia vs sham, p < 0.001) (Fig. 6B).

In serum UCH-L1 and GFAP concentrations, there were no significant differences between treatment groups in the ischemia and reperfusion phase (Fig. 6C and D).

Discussion

Early Induced Hypothermia Reduces Neuronal Degeneration and Injury Volume After Acute SDH in a Rat Model

We have shown that early induced, prereperfusional hypothermia was associated with reduced neuronal degeneration and injury volume when compared with normothermia or late hypothermia therapy in this acute SDH decompression rat model, based on Fluoro-Jade B–positive cell counts (Fig. 4) and injury volumetry (Fig. 5).

The pathology of ischemia/reperfusion injury can be separated into 2 mechanisms that play out over time. The ischemia-induced cellular dysfunction is followed by reperfusion-induced free radical production.57,72 Reperfusion following ischemia results in a short period of excessive free radical production. Experimental measurements of post–ischemia/reperfusion free radical production demonstrate that oxygen- and carbon-centered free radical production peaks within 5 minutes of reperfusion9 and that hydroxyl generation peaks within 15 minutes.35 Thus, mitochondrial free radical production is an impor-

![Fig. 4.](https://example.com/fig4.jpg) Quantification of degenerating cells with Fluoro-Jade B (FJB) staining. A–C: Images of Fluoro-Jade B staining cells in the ipsilateral (injured) cortex (−3.2 mm from bregma) in the normothermia (A), late induced hypothermia (B), and early induced hypothermia (C) groups. Bar = 50 µm. D–F: Ipsilateral hippocampus images −3.2 mm from bregma (CA1–3, and dentate gyrus) in each treatment group. Bar = 500 µm. G: Fluoro-Jade B–positive cell counting in each region (mean ± SEM). In the normothermia group, many degenerating cells were observed in all groups, especially in the ipsilateral cortex. Early induced hypothermia appears to mitigate the ischemia/reperfusion injury. #p < 0.05 vs E-Hypo; @p < 0.01 vs E-Hypo; *p < 0.01 vs Sham; **p < 0.001 vs Sham. Contra = contralateral; Ipsi = ipsilateral.
tant target and provides the first theoretical “window of opportunity” for hypothermia treatment. We speculate that intraischemic cooling was beneficial in our model because it achieves lower blood temperature at the time of reperfusion (see Fig. 3).

A second window of opportunity for hypothermia targets the inflammatory cascade and cell death pathways of apoptosis and necrosis, which is initiated by reperfusion. These probably center on mitochondrial dysfunction, and previous data have suggested that a transition in mitochondrial permeability may be the “point of no return” in both cell death pathways. Apoptosis is adenosine triphosphate dependent, whereas necrosis is not. Activation of caspases and proteases, and the release of mitochondrial cytochrome c, are features of apoptosis. These cell death processes represent the second “therapeutic opportunity” for hypothermia.

Despite much basic and clinical research concerning ischemia/reperfusion brain injury, the mechanisms of neuronal protection in hypothermia therapy remain unknown. Therapeutic hypothermia is believed to confer protection against ischemia/reperfusion injury through multiple mechanisms, such as by reducing cellular metabolism and oxygen demand while maintaining acceptable adenosine triphosphate levels.

Additionally, hypothermia attenuates abnormal free radical production, improves cellular ion handling, and improves cellular pH balance. Hypothermia also reduces cell death and inflammatory signaling. Although different tissues have different sensitivities to ischemia, ischemia/reperfusion injury has been observed in many tissue types.

In our results, we were able to show the beneficial effects of early induced hypothermia on brain damage associated with acute SDH. Early mild hypothermia may reduce reperfusion neuronal damage after decompression of focal mass in acute SDH rat model by reducing neurotoxic mechanisms, including oxidative stress, free radical generation, and vascular perturbations. Based on these data, it can be proposed that hypothermia should be initiated before reperfusion injury whenever possible.

Fig. 5. TTC volumetry in each treatment group. A: Examples of the TTC injury area in each treatment group, 6 mm from the tip (± 1.0 mm from bregma). In TTC staining, ischemic injuries were identified as unstained, white areas (asterisks). In the sham-treated rat, we could not find any ischemia in these photographs. However, in the late induced hypothermia and normothermia groups, we could identify large ischemic lesions compared with the early hypothermia group. Bar = 5 mm. B: Bar graph showing the ischemic areas in each section (mean ± SEM). In the early induced hypothermia group, the injury volume was smaller across all sections. C: Bar graph showing ischemic volume. In the early hypothermia group, the ischemic area was significantly smaller than in the late hypothermia and normothermia groups (’p < 0.05).

Early Induced Hypothermia Attenuates Both Neuronal and Glial Cell Damage in the Reperfusion Phase of Ischemia/Reperfusion Brain Injury

We observed that concentrations of UCH-L1 and GFAP in microdialysate were low only in the early induced hypothermia group. As far as we know, this is the first study that demonstrated the utility of UCH-L1 and GFAP as measured by microdialysis as biomarkers of acute SDH-induced brain damage and measures of moderate therapeutic hypothermia efficacy.

As shown in Fig. 6, the peak of UCH-L1 extracellular concentration was higher in the normothermia treatment group than in the sham group. This indicates that the peak of neuronal injury occurs in the early reperfusion phase of acute SDH (Fig. 6A). Also, the peak of the extra-
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cellular GFAP concentration in the normothermia group was significantly higher than that in the sham group and occurs in the late phase of reperfusion (normothermia, 15.5 ± 8.4 ng/dl; sham, 3.3 ± 1.0 ng/dl; normothermia vs sham, p < 0.01) (Fig. 6B).

The peak of the extracellular concentration of UCH-L1 and subsequent peak of GFAP seemed to be lowered by early hypothermia induction (Fig. 6A and B). However, late induced hypothermia could not attenuate the neuronal damage in the early phase of reperfusion but reduced only the subsequent glial damage as determined by GFAP in the late phase of reperfusion. Thus, taken together, these data suggest that early induced hypothermia could reduce neuronal and subsequent glial injury in the delayed reperfusion phase of ischemia/reperfusion injury or an astrocytic response to neuronal death. In hypoxic-ischemic models of brain damage, neurons have been shown to be much more sensitive and vulnerable than astrocytes. Astrocytes also might be more tolerant than neurons to ischemia/reperfusion neurotoxicity, as seen in this acute SDH rat model.

In the serum samples, we were unable to detect any significant difference between the ischemic and reperfusion phases in the early versus late hypothermia treatment groups (Fig. 6C and D). Papa et al. reported that high UCH-L1 concentrations in the CSF were highly related to injury severity and outcome in TBI in humans. The UCH-L1 concentrations were in the range of 10–100 ng/dl in the CSF samples from humans, consistent with those results in our microdialysis data. Furthermore, we also observed that extracellular concentrations of UCH-L1 and GFAP were 10–100 times higher in microdialysate than those in serum (Fig. 6). We think that microdialysis affords a direct measure of conditions within and around injured tissue, and our microdialysis data support its use as a reliable biomarker, better than the CSF, and unaffected by the status of the BBB. Serum UCH-L1 concentration has been shown to correlate directly with the ratio of albumin in the serum to that in the CSF, implying that efflux of UCH-L1 into the blood would only be possible when the BBB was open. Thus, serum UCH-L1 levels might not directly indicate the severity of primary and secondary brain injury, because without BBB permeability, efflux of the biomarker may be much reduced.

Another explanation for this discrepancy might be the difference in blood sampling time. In a recent report relating to serum UCH-L1 concentration, blood sampling was performed within 24 hours after injury. In a hypothermic cardiac arrest study in dogs, serum UCH-L1 concentration peaked at 8 hours after cardiac arrest in heart surgery. In our protocol, however, the blood sampling timing might be too early after injury, and thus the serum UCH-L1 concentration might peak at a later time. An additional serum sample in the late phase after injury could have helped identify the serum UCH-L1 peak.

Appropriate Timing and Duration of Hypothermia Therapy

The appropriate and effective timing of hypothermia induction in brain injury remains controversial. Previous studies have shown that hypothermia should be achieved within 2–6 hours of severe hypoxic-ischemic injury in sheep, gerbils, and rats to afford protection. For example, cooling sheep to 34°C for 72 hours gave good protection if started 90 minutes after the injury, was partly effective if started at 5.5 hours, and ineffective if started at 8.5 hours. On the other hand, some experimental reports with delayed induction of therapeutic hypothermia in an ischemic rat model also exist. In the study by Colbourne and Corbett, 48-hour period of mild hypothermia was induced starting 6 hours after a 10-minute-long severe 4-vessel occlusion ischemia in rats. Untreated normothermic ischemia resulted in total CA1 cell loss (99%), whereas delayed hypothermia treatment reduced neuronal loss to 14% at a 28-day survival. These results also indicate that the potential of late but prolonged hypothermia in the reperfusion phase might be effective in the ischemia/reperfusion rat model. In our study, late induced hypothermia did not reduce early ischemia/reperfusion injury (Fig. 5). However, in our study, the duration of hypothermia was short, only 3 hours. Based on these results, more prolonged hypothermia treatment in an ischemia/reperfusion acute SDH model may be useful to determine the minimum hypothermia duration that is beneficial.
Results of Multicenter Clinical Trials of Hypothermia and Their Interpretation

In a multicenter trial of hypothermia for neuroprotection,12 392 patients with acute brain injury were randomized to normothermia or surface-induced hypothermia; hypothermia did not improve outcome.12 However, there was weak evidence of improved outcomes in patients who were hypothermic on admission and were treated with continued hypothermia.12 This same group then tried to confirm the efficacy of very early hypothermia in patients with severe brain injury with the NABISH II trial.13 In the NABISH II, early induced hypothermia did not show efficacy as judged from mortality and morbidity data. On the other hand, in a post hoc, subpopulational analysis separating patients with diffuse brain injury and those with surgical hematoma evacuation, early induced hypothermia appeared efficacious for the hematoma evacuation group. The authors thus concluded that one explanation was the different pathophysiology between diffuse brain injury and hematoma. In experimental models, ischemia occurs during acute SDH expansion and is followed by reperfusion after surgical removal of the hematoma.38 This is similar to the pathophysiology seen in patients with cardiac arrest, a group that has been successfully treated with hypothermia.26

Experimentally, intraischemic hypothermia prior to hematoma removal is associated with improved outcome.11 Diffuse brain injury is not characterized by ischemia in laboratory studies, and thus patients with this condition may not be good candidates for hypothermia treatment. In consideration of these clinical data, we decided to test early induced hypothermia in the setting of an acute SDH model in rats, which would simulate ischemia/reperfusion injury to the brain. Supported by our present data, a further clinical trial, targeted on focal mass/decompression injury, is warranted to investigate the effect of early hypothermia in ischemia/reperfusion TBI, such as SDH. Several researchers have pointed out that the cooling rate, period of hypothermia, rewarming rate, and volumes of intravenous fluid for such a clinical trial are critically important variables.25,53,58,69

Limitations and Future Implications

Some of the limitations of our current study must be considered. First, microdialysis is only a regional technique and cannot assess global brain damage with certainty. The microdialysis technique might be good at time-dependent analysis; however, microdialysis might be weak for spatial injury analysis. In this study, we applied Fluoro-Jade B counting and TTC staining for this limitation. When we use a microdialysis technique as a monitor in clinical neurointensive care, additional data that represent global condition (for example, intracranial pressure monitoring, frequent CT examination, Xe-CT cerebral blood flow mapping, PET, or MRI) will be needed. The second limitation of this study was the natural rewarming from the hypothermia, due to technical constraints, rather than inducing a slow rewarming phase, as would be done in a clinical study.13 Third, our outcome assessments were limited to biomarker, histopathological, and volumetric end points. In a clinical study, however, functional outcome (for example, Extended Glasgow Outcome Scale) is a more informative measure.

Conclusions

Early induced hypothermia can reduce neuronal degeneration and injury volume and attenuate both neuronal and glial cell damage in this acute SDH/decompression rat model. Our results support a clinical, multicenter trial to further examine the efficacy of very early, preoperatively induced hypothermia, in this tightly related acute SDH/trumatic intracerebral hemorrhage subgroup of patients with poor outcome.

Disclosure

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