TREATMENT WINDOW FOR HYPOThERMIA IN BRAIN INJURY

CARRIE G. MARGRAF, PH.D., GUY L. CLIFTON, M.D., AND MELANIE R. MOODY, M.S.

Vivian L. Smith Foundation for Neurologic Research and Department of Neurosurgery, The University of Texas Health Science Center at Houston, Texas

Object. The goal of this study was to evaluate the therapeutic window for hypothermia treatment following experimental brain injury by measuring edema formation and functional outcome.

Methods. Traumatic brain injury (TBI) was produced in anesthetized rats by using cortical impact injury. Edema was measured in the ipsilateral and contralateral hemispheres by subtracting dry weight from wet weight, and neurological function was assessed using a battery of behavioral tests 24 hours after TBI. In injured rats, it was found that brain water levels were elevated at 1 hour postinjury, compared with those in sham-injured control animals, and that edema peaked at 24 hours and remained elevated for 4 days. Hypothermia (3 hours at 30°C) induced either immediately after TBI or 60 minutes after TBI significantly reduced early neurological deficits. Delay of treatment by 90 or 120 minutes postinjury did not result in this neurological protection. Immediate administration of hypothermia also significantly decreased the peak magnitude of edema at 24 hours and 48 hours postinjury, compared with that in normothermic injured control animals. When delayed by 90 minutes, hypothermia did not affect the pattern of edema formation.

Conclusions. When hypothermia was administered immediately or 60 minutes after TBI, injured rats showed an improvement in functional outcome and a decrease in edema. Delayed hypothermia treatment had no effect on functional outcome or on edema.

KEY WORDS • traumatic brain injury • impact injury • edema • hypothermia • rat
All rats were allowed to survive for 24 hours and were given a battery of functional tests to assess early neurological outcomes.

**Experiment III: Effect of Hypothermia on Edema Following Moderate TBI**

One hundred rats were used in this experiment and assigned to groups. The names of the groups indicate the type of treatment the rats received. Thirty-two animals were assigned to each of the following groups: TBI–normothermia group, TBI–immediate hypothermia group, and TBI–90-minute delayed hypothermia group. These animals were divided into subgroups of four animals each and were killed at the following time points: 1, 1.5, 6, 24, or 48 hours or 4, 7, or 10 days after injury. After death, the brains of the animals were examined and the extent of edema was assessed. An additional control group of four sham-injured normothermic animals were killed 24 hours after the sham injury and their brains were also examined.

**Cortical Impact Injury Procedure**

The TBI was produced in anesthetized male Sprague–Dawley rats by using the controlled cortical impact injury method. The rats were anesthetized with isoflurane in a 1:1 O₂/N₂O atmosphere. A midline incision was made in the scalp and the skull was exposed. A 7-mm-diameter craniotomy was made midway between bregma and lambda, overlying the temporoparietal cortex on the right side. Injury was produced using a pneumatic impactor with a piston velocity of 4.5 m/second, a dwell time of 50 msec, and a penetration depth of 1.6 mm—parameters previously shown to produce a reliable yet treatable injury. Following injury, the wound was closed with wound clips. Sham injury was performed by conducting all of the aforementioned surgical procedures, except the impact injury. Any animal that died between injury and the scheduled time of death was excluded from analysis.

**Hypothermia Treatment**

Hypothermia (30°C) induction was begun either within 5 minutes or within 90 minutes of TBI by wrapping the anesthetized rat in a flexible cold pack, as we have previously described. The rats were maintained in an anesthetized state by administration of 1% isoflu ranate throughout treatment (1:1 O₂/N₂O) to keep them immobilized. The temperature of the right temporalis muscle and the core temperature, which was measured with a rectal probe, were monitored during cooling. When the rat’s temporalis muscle temperature reached the target temperature of 30°C, the 3-hour hypothermia period began. The rats cooled rapidly and there was little variation between animals (15 ± 3 minutes from initiation of cooling to reach 30°C). The time spent to reach the target temperature was recorded for each rat. Core temperature was recorded at 15-minute intervals during the period in which the targeted temperature was maintained. Adjustments were made using either a heating pad or by reapplication of the ice pack to keep the rat at 30 ± 1°C. After 3 hours, rewarming was accomplished by placing a heating pad under the rat and monitoring the temperature so that the rat returned to normothermia (37°C) steadily over a 2-hour period. Rats assigned to a normothermic group were maintained on anesthesia at 37°C for 5 hours and their core temperatures were recorded every 15 minutes.

**Measurement of Edema**

At the assigned time point, the rat was killed by CO₂ and decapitated. The brain was removed from the skull and the cerebellum and brainstem were discarded. The remaining brain was divided in half longitudinally by using a razor blade. Each half was weighed to determine wet weight and then dried in an oven at 100°C for 24 hours. The brain was again weighed after full drying to determine dry weight. Edema was calculated using the following formula: (wet weight in g – dry weight in g)/wet weight in g × 100.

**Behavioral Testing**

Twenty-four hours after injury, neurological function was assessed using three tests—balance-beam performance, posture reflex, and vertical screen. An investigator who remained blinded to each rat’s group assignment conducted the behavioral testing. Following behavioral testing, the rats were killed.

**Balance-Beam Test**

Vestibular function was evaluated while the animal was on the balance beam, a narrow elevated wooden beam 30.5 cm long × 1.5 cm wide, which was located 27 cm above the bench top. The rats were placed on the beam with the head facing away from the wall, and were allowed to remain on the beam for 60 seconds. Duration on the beam was recorded for a maximum of 60 seconds. Each rat was given three trials, and a mean score was determined following the procedures described by Dixon and colleagues.

**Posture-Reflex Test**

This variation of the posture-reflex test, described by Bederson and colleagues, is used to evaluate asymmetry of the forelimbs as the rat is suspended by the base of the tail. Normal posture is defined as symmetrical reaching of both forelimbs toward the ground, and was given a score of 3. Moderately abnormal posture, demonstrated by flexion of the contralateral forelimb toward the body, was given a score of 2. Severely impaired posture, characterized by flexion of the forelimb and rotation of the shoulder and/or body, was given a score of 1.

**Vertical-Screen Test**

To measure muscle strength, we used a wire mesh screen (29.5 cm × 59.5 cm with grid openings measuring 2 mm²) that could be rotated. The rats were placed on the horizontal screen, and the screen was rotated to a vertical plane for 5 seconds. The rats were scored for their ability to grip onto the screen. Neurologically intact rats could grip the screen for the full 5 seconds and their efforts were assigned a score of 4. Slipping but still gripping was assigned a score of 3; slipping and falling off, a score of 2; and falling immediately off the screen, a score of 1.

**Statistical Analyses**

Group means ± standard errors of the mean were calculated for edema of the contralateral and ipsilateral sides for each time point, and for each behavioral test for each group. The data obtained in Experiment I were compared using the Kruskal–Wallis test (H), followed by the Dunn test for paired comparisons wherever appropriate, because centrality analysis showed that the data were not normally distributed. Behavioral data from Experiment II were analyzed using ANOVA (balance beam) or the Kruskal–Wallis test (posture reflex and vertical screen). For Experiment III, group means were compared using ANOVA, followed by Fisher tests for paired comparisons wherever necessary.

**Results**

**Experiment I: Time Course of Edema Following Moderate TBI**

As seen in Fig. 1, edema was only observed in the ipsilateral hemisphere following injury. The Kruskal–Wallis test showed that there were no differences over time for tissue of the contralateral hemisphere (H = 2.21 [df 7], p > 0.05). On the ipsilateral side, there was a significant effect of group (H = 11.375 [df 7], p < 0.05), with post hoc comparisons demonstrating that significant edema was present 1 hour after injury, increased at 24 hours after injury, remained elevated for 4 days, and decreased by 7 days, although not to uninjured levels.

**Experiment II: Therapeutic Window of Hypothermia Treatment**

The therapeutic window for administering hypothermia was evaluated at the following four time points: immediately following injury (0-minute delay), 1 hour after injury (60-minute delay), 90 minutes after injury (90-minute delay), and 2 hours after injury (120-minute delay). Each treated group was compared with a normothermic group as
well as with a sham-injured control group assigned to the same treatment delay groups.

Temperature During the Delay. During the delay, the animals were returned to their home cages and allowed to awaken. Body temperature was recorded every 15 minutes for all groups. Immediately after anesthesia wore off, rats in all groups were slightly hypothermic (mean 35.8 ± 0.15°C), but all recovered by the first 15-minute reading and were maintained at normothermic temperatures until induction of hypothermia.

Results of Balance-Beam Test. The mean balance-beam performance for all the groups is depicted in Fig. 2 upper. It is evident that rats in the sham groups all performed well, remaining on the beam during the entire 60-second trial period and that rats in each of the untreated TBI-normothermia groups had significantly impaired ability to complete the task. The rats in the group in which hypothermia was administered immediately after TBI (0-minute delay hypothermia group) displayed significantly improved balance-beam performance compared with those in the 0-minute delay normothermia group, according to one-way ANOVA (F2,38 = 16.45, p < 0.001) and post hoc comparisons. Similarly, hypothermia initiated 60 minutes after TBI significantly improved balance-beam performance compared with normothermia initiated 60 minutes after TBI, as shown by ANOVA (F2,38 = 46.33, p < 0.001) and post hoc comparisons. When administration of hypothermia was delayed by 90 or 120 minutes, there was no effect on balance-beam performance, although animals in both injured groups differed from sham-injured animals (90-minute delay hypothermia group: F2,38 = 36.05, p < 0.05; 120-minute delay hypothermia group: F2,38 = 23.56, p < 0.05).

Results of Posture-Reflex Test. Results of the posture-reflex test paralleled those of the balance-beam test. The results for all groups are shown in Fig. 2 lower. The animals in the sham-injury groups all performed perfectly and those in the untreated TBI–normothermia groups all exhibited significant deficits compared with those in the uninjured control groups. Hypothermia administered immediately attenuated the posture-reflex deficit (F2,38 = 16.39, p < 0.001), according to ANOVA and post hoc comparisons. Similarly, ANOVA and post hoc comparisons demonstrated that rats in which hypothermia was delayed 60 minutes had significantly reduced posture-reflex deficits (F2,38 = 14.52, p < 0.001) compared with those in the untreated normothermia group. Analysis by ANOVA showed that delaying hypothermia treatment for 90 or 120 minutes rendered the treatment ineffective, although animals in all injured groups exhibited impairments compared with those in sham-injured control groups (90-minute delay hypothermia group: F2,30 = 9.23, p < 0.05; 120-minute delay hypothermia group: F2,30 = 8.57, p < 0.05).

Vertical-Screen Test. The vertical-screen test was not sensitive to hypothermia treatment for any of the conditions. Rats in the sham-injury groups all performed well on the screen (mean scores 3.75 ± 0.25, 3.63 ± 0.18, 4 ± 0, and 3.88 ± 0.13 for the 0-, 60-, 90-, and 120-minute delay...
Experiment III: Effect of Hypothermia on Edema Following Moderate TBI

Figure 3 demonstrates the effect of hypothermia applied immediately (upper) or delayed by 90 minutes (lower) on edema formation following TBI. As observed in Experiment I, edema was not found in the contralateral hemisphere following injury ($F_{1,6} = 1.556$, not significant) and there was, thus, no effect of treatment ($F_{3,18} = 5.981$, not significant), as shown by ANOVA. Edema increased significantly after injury ($F_{1,6} = 17.138$, $p < 0.001$) in the ipsilateral hemisphere. Post hoc comparisons showed that immediate application of hypothermia significantly decreased the peak magnitude of edema (24 and 48 hours), compared with animals maintained at normothermic temperature. When the same hypothermic treatment was delayed by 90 minutes, there was no effect on edema formation, compared with normothermic treatment ($F_{3,12} = 0.355$, not significant).

Discussion

We report that hypothermia therapy applied immediately following brain injury in the rat decreases the magnitude of the peak edema response and confers protection on neurological function. Edema was significantly decreased at 24 and 48 hours after injury in animals that were given 3 hours of hypothermia immediately after injury. Edema did not appear to be reduced during the time of treatment. When the same hypothermia treatment was applied after a delay of 90 minutes, however, there was no effect on the pattern of edema formation and there was no effect on behavioral outcome.

The time course of edema formation in this model parallels the course of ICP rise demonstrated by the Traumatic Coma Data Bank. In rats, as in humans, edema is observed within the 1st hour after brain injury in the rat. This edema peaks at 24 hours and remains elevated for 4 days. At 7 and 10 days postinjury, it has decreased, although not to the level of uninjured tissue. These data suggest that this model may have clinically relevant characteristics.

It is currently impossible to know how the therapeutic window for hypothermia in rodents relates to a putative therapeutic window in brain-injured humans. From the present data, however, it may be concluded that if hypothermia is to be effective in humans, the window for treatment is probably very brief. Clinical data supporting this supposition can be found in the recently completed NABISH clinical trial. The NABISH was used to evaluate the safety and efficacy of whole-body hypothermia ($32–33^\circ C$ for 48 hours) compared with normothermia ($37^\circ C$ for 48 hours) in 392 patients with severe brain injury (Glasgow Coma Scale scores 3–8). In that trial, induction of hypothermia began an average of 4.3 ± 1 hours after injury and the target temperature was reached at a mean of 8.3 ± 3 hours after injury, according to the design of the trial. Post hoc analyses identified a subset of 88 patients who displayed hypothermia at admission ($<35^\circ C$), and who were younger than 45 years, who, when treated with hypothermia, showed a statistically significant increase in dichotomized Glasgow Outcome Scale findings (fair compared with poor outcome) at 6 months, compared with those patients who were randomized to the normothermia group. In those patients who displayed normothermia at admission, even reaching $33^\circ C$ by 5 hours after injury was not beneficial.

Achieving $33^\circ C$ earlier than 5 hours postinjury in patients poses logistical challenges. The data obtained during the NABISH trial suggest what may be the practical limits...
Hypothermia treatment window

to very early cooling.6 Resuscitation in the emergency department requires approximately 1 hour, and thus patients in NABISH were admitted to the hospital 1.3 ± 0.9 hours after injury. With surface cooling, a rate of 2°C/hour is achievable. Thus, optimally, patients could reach the target temperature of 33°C from their average temperature of 36°C by 4.5 hours after injury. Achieving hypothermia earlier would require initiation of cooling in the ambulance or use of intravascular procedures.

The finding here that hypothermia reduces edema is consistent with other laboratory data. It is known that brain edema is associated with a breakdown in the blood–brain barrier2 and that hypothermia can acutely reduce blood–brain barrier breakdown.12 Hypothermia has also been shown to reduce vasogenic edema caused by cold-induced lesions of the cortex in rats14,20 and to decrease edema caused by experimental subdural hematoma.14 In the NABISH trial, hypothermia significantly reduced elevated ICP during treatment, even in patients for whom there was no functionally protective effect of treatment at 6 months, suggesting that any neuroprotective effect of hypothermia in humans may be independent of its ICP reduction effects.

Conclusions

We have shown that the effect of hypothermia on brain edema following TBI cannot be separated from its neuroprotective effects, and that the window after injury during which we can intervene may be short.

References


Manuscript received February 28, 2000.
Accepted in final form August 14, 2001.
Address reprint requests to: Carrie G. Markgraf, Ph.D., Schering-Plough Corporation, P. O. Box 32, 144 Route 94, Lafayette, New Jersey 07848. email: carrie.markgraf@spcorp.com.