Therapeutic hypothermia preserves antioxidant defenses after severe traumatic brain injury in infants and children*

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Objective: Oxidative stress contributes to secondary damage after traumatic brain injury (TBI). Hypothermia decreases endogenous antioxidant consumption and lipid peroxidation after experimental cerebral injury. Our objective was to determine the effect of therapeutic hypothermia on oxidative damage after severe TBI in infants and children randomized to moderate hypothermia vs. normothermia.

Design: Prospective randomized controlled study.

Setting: Pediatric intensive care unit of Pittsburgh Children's Hospital.

Patients: The study included 28 patients.

Measurements and Main Results: We compared the effects of hypothermia (32°C–33°C) vs. normothermia in patients treated in a single center involved in a multicentered randomized controlled trial of hypothermia in severe pediatric TBI (Glasgow Coma Scale score ≤8). The patients randomized to hypothermia (n = 13) were cooled to target temperature within 6 to 24 hours for 48 hours and then rewarmed. Antioxidant status was assessed by measurements of total antioxidant reserve and glutathione. Protein oxidation and lipid peroxidation were assessed by measurements of protein thiols and F2-isoprostane, respectively, in ventricular cerebrospinal fluid (CSF) samples (n = 76) obtained on day 1–3 after injury. The association between Glasgow Coma Scale score, age, gender, treatment, temperature, time after injury, and CSF antioxidant reserve, glutathione, protein-thiol, F2-isoprostane levels were assessed by bivariate and multiple regression models. Demographic and clinical characteristics were similar between the two treatment groups. Mechanism of injury included both accidental injury and nonaccidental injury. Multiple regression models revealed preservation of CSF antioxidant reserve by hypothermia (p = 0.001). Similarly, a multiple regression model showed that glutathione levels were inversely associated with patient temperature at the time of sampling (p = 0.002). F2-isoprostane levels peaked on day 1 after injury and were progressively decreased thereafter. Although F2-isoprostane levels were approximately threefold lower in patients randomized to hypothermia vs. normothermia, there was no statistically significant difference.

Conclusion: To our knowledge, this is the first study demonstrating that hypothermia attenuates oxidative stress after severe TBI in infants and children. Our data also support the concept that CSF represents a valuable tool for monitoring treatment effects on oxidative stress after TBI. (Crit Care Med 2009; 37:689–695)

Key Words: pediatric head injury; biomarkers of oxidative stress; chemiluminescence; fluorescence

Mild to moderate therapeutic hypothermia (32°C–33°C) has been used in clinical practice in pediatric patients with severe traumatic brain injury (TBI), specifically as a second-tier therapy (1, 2). Two recent studies, including a multicenter trial, have demonstrated safety of this treatment modality after TBI in children, along with beneficial effects on intracranial hypertension (3, 4). Neuroprotective effects of hypothermia have been shown in a variety of animal species and mechanisms of injury (5–9), and clinical trials have reported efficacy in both cardiac arrest in adults (10, 11) and perinatal asphyxia in newborns (12). Several secondary injury mechanisms are favorably influenced by moderate hypothermia in experimental TBI (13–15). However, the precise mode of neuroprotective action of mild–moderate hypothermia is not known.

Moderate hypothermia has been shown to have beneficial effects on oxidative stress in experimental models of TBI. Generation of hydroxyl radicals as analyzed by salicylate-trapping method was attenuated by moderate hypothermia after fluid percussion injury in rats (16). Furthermore, treatment of rats with moderate hypothermia after controlled cortical impact increased superoxide dismutase activity relative to values in injured normothermic animals (13). Similarly, therapeutic hypothermia has been shown to attenuate consumption of endogenous antioxidants and decrease lipid peroxidation in experimental temporary focal ischemia and cardiac arrest (14, 15).

Assessment of the extent of oxidative stress in vivo is a complex task requiring usage of a battery of assays evaluating...
radical scavenging capacity and oxidation products of biomolecules. We previously presented evidence for free radical-mediated lipid peroxidation (by assessment of P2-isoprostanes) and protein oxidation (by assessment of protein thiol) and sustained decreases in total antioxidant reserve and glutathione concentrations in cerebrospinal fluid (CSF) after severe TBI in infants and children. P2-isoprostanes are bioactive cyclopentanone prostaglandin-like compounds produced in vivo by free radical peroxidation of arachidonyl-containing lipids and represent a reliable lipid biomarker of oxidative stress (17). Free radical attack on proteins results in oxidation of their sulfhydryl groups, leading to decreased protein thiol concentrations. Our findings suggested that these CSF markers could be valuable to assess the effect of therapies on oxidative stress after TBI in patients.

Several studies suggest that CSF oxidation markers might be associated with outcome after TBI. Pilitsis et al (16) demonstrated that elevated levels of highly oxidizable polysaturated fatty acids in CSF were associated with worse outcome in adults with severe TBI. Enhanced lipid peroxidation, as assessed by CSF thiobarbituric acid reactive substances, was reported to correlate with the severity of head injury in adults with contusion (18). In a recent study, Darwish et al (19) showed that poor neurologic outcome was associated with increased levels of nitrotyrosine, a marker of protein damage by oxidative/nitrative stress, in CSF after TBI in adults. These studies indicate that CSF markers of oxidative stress may be useful in prognostication after TBI.

To date, there has not been any study assessing the effects of hypothermia on oxidative stress after clinical TBI in either adults or children. Therefore, in this study, we tested the hypothesis that therapeutic hypothermia attenuates oxidative damage as assessed by markers of lipid peroxidation, protein oxidation, and antioxidant status (reduced glutathione and total antioxidant reserve) in CSF after severe TBI in infants and children.

METHODS

Patient Selection and Data Collection. We examined the effect of moderate therapeutic hypothermia on oxidative stress in CSF from subsets of patients (n = 28) enrolled at our center in two concurrent randomized controlled trials assessing the effect and safety of moderate therapeutic hypothermia in severe TBI (Glasgow Coma Scale [GCS] score ≤8) in infants and children. The general paradigm for patients treated with hypothermia involved cooling to 32°C–33°C (within either 6 or 24 hours after injury) for 48 hours and then gradual rewarming. The details of the study protocols and results of these trials on clinical outcome have been previously reported (3). Briefly, once the patient was randomized to normothermia or hypothermia, a temperature control unit with a rectal probe was used for surface cooling or warming as needed. Temperature was maintained by means of a rectal probe at 32°C–33°C for hypothermia and at 36.5°C–37.5°C for normothermia for the 48-hour study period. To prevent shivering, which could make cooling difficult, sedation and paralysis were used before the initiation of cooling (hypothermia group) and during the study period in both groups. Patients randomized to normothermia were maintained at 36.5°C–37.5°C throughout the study period and were passively warmed if their initial presenting core temperature was less than 36°C. After 48 hours of cooling, rewarming occurred by passively warming the patient 1°C every 4–4 hours so as to reach normothermia (36.5°C) within 12–18 hours (3). The predetermined criteria, in addition to closed head injury and a GCS score of ≤8, were age of 0–156 months (multicenter trial) or 0–18 years (single center trial). Patients were excluded from the study if they had a normal initial computed tomography scan (no blood, fracture, swelling, and/or shift), GCS score of 8 with a computed tomography scan with only minimal abnormal findings, prolonged hypotension (>15 minutes) defined as a mean blood pressure less than fifth percentile for age, failure to obtain informed consent within 6 hours of injury (multicenter trial), failure to obtain informed consent within 24 hours of admission to Children’s Hospital of Pittsburgh (single-center trial), brain dead clinically, penetrating cerebral injury, coagulopathy, prothrombin time >16 and partial thromboplastin time >40, or pregnancy. This study was approved by the Institutional Review Board of the Children’s Hospital of Pittsburgh, and informed consent was obtained from parents for sample collection. CSF samples (n = 76) were centrifuged for 10 minutes at 5000 × g and stored at −70°C until the time of analysis. Demographic and clinical parameters are seen in Table 1.

All patients with severe TBI admitted to the Children’s Hospital of Pittsburgh were treated with ventricular catheter insertion, and CSF was drained continuously. All patients were also intubated and mechanically ventilated to PaCO2 of 35–38 mm Hg. They received sedation with narcotics (fentanyl) and neuromuscular blockade with vecuronium bromide to maintain their intracranial pressure and cerebral perfusion pressure in the age-appropriate target range in accordance with the guidelines for management of severe pediatric TBI (1). Barbiturates and mechanical ventilation to PaCO2 <35 mm Hg were used as second-tier therapies as needed for refractory intracranial hypertension.

Chemiluminescence Measurements of Total Antioxidant Reserve. Total antioxidant reserve in CSF was assessed by chemiluminescence produced in the presence of luminol and a source of peroxyl radicals, as described by Tyurina and co-workers (20). A water-soluble azo-initiator, 2,2′-azo-bis(2-amidinopropane)-dihydriodlchloride (AAPH), was used to produce peroxyl radicals at a constant rate. Oxidation of luminol (400 μM) by AAPH-derived peroxyl radicals was assayed by monitoring the chemiluminescence response. The reaction was initiated by addition of AAPH. A delay in the chemiluminescence response, which is caused by interaction of endogenous antioxidants with AAPH-derived peroxyl radicals, was observed on addition of CSF. On the basis of the known rate of peroxyl radical generation by AAPH, the amount of peroxyl radicals scavenged by endogenous antioxidants was determined. A Microlite ML 1000 microtiter plate luminometer (Dynatech Labs, Chantilly, VA) was used for these determinations.

Fluorescence Assay of Protein Sulfhydryls and Glutathione. CSF protein sulfhydrys (prot-SH) and glutathione concentrations were measured by fluorescent assay using ThioGlo-1 (Convalex Associates, Woburn, MA), a maleimide reagent that produces a highly fluorescent product on its reaction with sulfhydryl groups, as described previously (21). A Cytofluor 2350 fluorescence plate reader (Millipore Corporation, Marlborough, MA) was used to detect fluorescence using

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypothermia</th>
<th>Normothermia</th>
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<tbody>
<tr>
<td>Age</td>
<td>6.8 ± 5.1</td>
<td>5.1 ± 5.4</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>9 (69)</td>
<td>8 (53)</td>
</tr>
<tr>
<td>Glasgow Coma Scale score</td>
<td>6.3 ± 1.3</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td>Mechanism of injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor vehicle injury</td>
<td>7 (54)</td>
<td>10 (66)</td>
</tr>
<tr>
<td>Inflicted traumatic brain injury</td>
<td>2 (15)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Fall</td>
<td>3 (23)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (8)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Values are means ± SD. Percentages are shown in parenthesis.
Excitation and emission wavelengths of 388 and 500 nm, respectively. The data acquired were exported from the spectrophotometer using Cytofluor software.

**Determination of F2-Isoprostane (8-epi-prostaglandin F2α).** CSF F2-isoprostane content was measured by a commercial enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) with a detection limit: 2 pg/mL.

**Statistical Analysis.** Data are shown as mean ± standard error of mean. Demographic and clinical data were compared by Student’s t test. The association between GCS score, age, gender, treatment (randomization to hypothermia or normothermia), time after injury, and CSF sampling instead of randomization to treatment times were not different between hypothermia or normothermia groups (10.4 ± 4.8 hours vs. 12.4 ± 7.3 hours on day 1; 42.5 ± 3.2 hours vs. 40.8 ± 4.4 hours on day 2, 62.6 ± 5.4 hours vs. 66.9 ± 4.1 hours on day 3). Mean ± SD). The luminol-enhanced chemiluminescence assay revealed a reduction in total antioxidant reserve in patients randomized to normothermia vs. hypothermia (117.9 ± 8.34 vs. 90.23 ± 5.96 on day 2 after TBI) (Fig. 1). Bivariate and multiple regression models revealed a highly significant effect of hypothermia on CSF total antioxidant reserve independent of age, gender, and initial GCS score (p = 0.002 and p = 0.001) (Table 2). Sustained depletion of total antioxidant reserve was suspected because the greatest decrease was observed on day 3, compared with day 1 and 2 (Fig. 1). There was an inverse relationship between temperature and CSF total antioxidant reserve after injury (p = 0.022) (Table 3).

**Glutathione.** Glutathione levels progressively decreased after the peak on day 1, similar to that previously reported (Fig. 2). Bivariate and multiple regression models revealed a tendency for higher glutathione levels in the hypothermia group compared to the normothermia group.

**RESULTS**

**Patient Demographics.** Demographic and clinical parameters of TBI patients are shown in Table 1. Age ranged from 2 months to 16 years. There were 13 patients randomized to hypothermia and 15 patients randomized to normothermia. Initial GCS score ranged between 3 and 8. Mechanism of injury included both accidental injury and child abuse. Five patients randomized to hypothermia and eight patients randomized to normothermia received barbiturates. One patient randomized to hypothermia and two patients randomized to normothermia underwent decompressive craniotomy. There was no statistically significant difference between the groups for demographic and clinical characteristics.

**Total Antioxidant Reserve.** For all biochemical analysis, mean sample collection times were not different between hypothermia and normothermia groups. The correlation within individuals, were used. Some of the study patients randomized to therapeutic hypothermia did not reach the target temperature at the time of CSF sampling. Similarly, some patients randomized to normothermia were hypothermic at admission. To assess the impact of actual core temperature on CSF F2-isoprostane level, we used a second model that included the actual core temperature at the time of CSF sampling instead of randomization to hypothermia or normothermia. In each of the models, the beta-coefficient represents the average increase or decrease in CSF biochemical marker levels for a one-unit increase in continuous variables (e.g., temperature) or the difference in the average CSF biochemical marker level between the two groups for dichotomous variables.

**Table 2.** Effect of hypothermia treatment on cerebrospinal fluid biomarkers of oxidative stress

<table>
<thead>
<tr>
<th>Cerebrospinal Fluid Biomarker</th>
<th>Bivariate Model</th>
<th>Multivariate Model</th>
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<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Total antioxidant reserve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (reference = NT)</td>
<td>−25.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Time (days 1, 2, 3)</td>
<td>−14.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0</td>
<td>0.79</td>
</tr>
<tr>
<td>Gender</td>
<td>−13.4</td>
<td>0.115</td>
</tr>
<tr>
<td>GCS</td>
<td>−6.4</td>
<td>0.385</td>
</tr>
<tr>
<td>Glutathione</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (reference = NT)</td>
<td>−0.3</td>
<td>0.097</td>
</tr>
<tr>
<td>Time (days 1, 2, 3)</td>
<td>−0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0</td>
<td>0.911</td>
</tr>
<tr>
<td>Gender</td>
<td>−0.1</td>
<td>0.79</td>
</tr>
<tr>
<td>GCS</td>
<td>0.1</td>
<td>0.645</td>
</tr>
<tr>
<td>Protein sulfhydryls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (reference = NT)</td>
<td>−6.1</td>
<td>0.063</td>
</tr>
<tr>
<td>Time (days 1, 2, 3)</td>
<td>0.1</td>
<td>0.969</td>
</tr>
<tr>
<td>Age</td>
<td>0</td>
<td>0.563</td>
</tr>
<tr>
<td>Gender</td>
<td>−4.6</td>
<td>0.151</td>
</tr>
<tr>
<td>GCS</td>
<td>−0.7</td>
<td>0.878</td>
</tr>
<tr>
<td>F2-isoprostane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (reference = NT)</td>
<td>13.3</td>
<td>0.099</td>
</tr>
<tr>
<td>Time (days 1, 2, 3)</td>
<td>−14.7</td>
<td>0.029</td>
</tr>
<tr>
<td>Age</td>
<td>0</td>
<td>0.913</td>
</tr>
<tr>
<td>Gender</td>
<td>3.6</td>
<td>0.668</td>
</tr>
<tr>
<td>GCS</td>
<td>5.4</td>
<td>0.381</td>
</tr>
</tbody>
</table>

GCS, Glasgow Coma Scale Score; NT, normothermia.

**Figure 1.** Effect hypothermia on cerebrospinal fluid (CSF) antioxidant reserves after traumatic brain injury. Hypothermia preserved antioxidant reserves after traumatic brain injury compared with normothermia. AAPH, 2,2′-azobis(2-aminopropane) dihydrochloride.
levels in CSF with hypothermia (p = 0.097 and p = 0.090) (Table 2). There was an inverse relationship between temperature and glutathione concentration in CSF after injury (p = 0.002) (Table 3).

Prot-SH Oxidation. CSF concentration of prot-SH was about three-fold lower on day 2 in patients randomized to hypothermia than in patients randomized to normothermia (9.45 ± 1.41 vs. 26.59 ± 7.21 nmol/mg protein) (Fig. 3). Bivariate and multiple regression models revealed a tendency for higher CSF prot-SH levels with hypothermia (p = 0.063 and p = 0.079) (Table 2). In general, there was an inverse relationship between temperature and CSF prot-SH levels at all times after injury, but this was not statistically significant (p = 0.104) (Table 3).

F2-Isoprostane. F2-isoprostane levels progressively decreased after the peak on day 1 in patients randomized to normothermia, similar to that previously reported (Fig. 4). Bivariate and multiple regression models did not reveal a significant effect of hypothermia (Table 2) despite 3.6-fold higher CSF F2-isoprostane levels in patients randomized to normothermia on day 1 after TBI (65.70 ± 24.83 pg/mL) compared with patients randomized to hypothermia (18.23 ± 1.84 pg/mL) (Fig. 4). At all times after injury, there was no significant temperature effect on CSF F2-isoprostane levels (p = 0.104) (Table 3).

DISCUSSION

We have previously shown that severe TBI in infants and children is accompanied by marked and progressive compromise of antioxidant defenses and free radical-mediated lipid peroxidation (21). Here, we present data demonstrating a beneficial effect of hypothermia on oxidative stress in the same setting. Our clinical data are consistent with results in experimental trauma models (13, 22) in that hypothermia attenuates consumption of endogenous antioxidants. In addition, we have shown for the first time that hypothermia tended to decreased protein oxidation. We observed an early peak in F2-isoprostane levels, consistent with our previous study in infants and children (21), suggesting the possible need for early application of therapies targeting some aspects of oxidative stress after TBI, such as lipid peroxidation.

Possible Mechanisms of Beneficial Effect of Hypothermia on Oxidative Stress after TBI. Mild–moderate hypothermia has been shown to have neuroprotective effects in experimental and clinical brain injury resulting from trauma, cardiac arrest, and ischemia (10, 11, 14, 15, 22, 23). The precise mode of neuroprotective action by mild–moderate hypothermia is not known. Most likely, mild–moderate hypothermia exhibits multiple and synergistic effects on brain metabolism; however, it does not indiscriminately slow down all biochemical cascades. There is controversy regarding effects of mild–moderate hypothermia on cerebral energy metabolism likely because of differences in experimental insults and species studied. Mild–moderate hypothermia has been shown to decrease the global cerebral metabolic rate for glucose and oxygen but maintain a slightly better energy level by reducing adenosine triphosphate breakdown (24, 25). Beneficial effects of mild–moderate hypothermia on energy balance and production of reactive oxygen species in mitochondria have also been reported after experimental ischemia reperfusion injury outside the central nervous system and in retina (26–

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Table 3. Association of cerebrospinal fluid metabolites with temperature

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total antioxidant reserve</td>
<td>-0.3</td>
<td>0.022</td>
</tr>
<tr>
<td>Protein sulfhydrys</td>
<td>-0.2</td>
<td>0.104</td>
</tr>
<tr>
<td>Glutathione</td>
<td>-0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>F2-isoprostane</td>
<td>-0.2</td>
<td>0.104</td>
</tr>
</tbody>
</table>

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Figure 2. Effect of hypothermia and time on cerebrospinal fluid glutathione (GSH) levels after traumatic brain injury.

Figure 3. Effect of hypothermia and time on cerebrospinal fluid protein sulfhydryl levels after traumatic brain injury. SH, sulfhydryl.
TBI, however, are not consistently atten-
(47). Increases in cytokines after severe
inducible nitric oxide synthase activity
senger RNA up-regulation (45, 46), and
cumulation (42–44), interleukin-1 mes-
tidenced by reductions in neutrophil ac-
are also favorably affected by therapeutic
matory response to cerebral contusion
Several components of the local inflam-
tantly linked to inflammatory response.
Moderate hypothermia has been shown to
brate accumulation as assessed by CSF
quino!nic acid levels was not attenuated
by moderate hypothermia in adult severe
TBI victims (50).
A beneficial effect of hypothermia on
oxidative stress has been shown in exper-
mental studies outside the central ner-
vous system with reduction in lipid per-
oxidation in ischemic kidney and liver
tissue (51, 52). Antioxidant supplementa-
tion with hypothermia had additive pro-
tective effects against lipid peroxidation
in these experiments. Similarly, our data
show that hypothermia only partially re-
ostored antioxidant defenses. Given that
mild–moderate hypothermia has shown
variable or partial beneﬁcial effects (in-
tracranial pressure vs. outcome) in clin-
cal TBI (4, 23, 53, 54), supplementation
with antioxidants may represent a valu-
able adjunct to hypothermia in the treat-
ment of TBI victims.
Clinical Implications. To date, there
have been no studies assessing the effect
of therapeutic hypothermia on oxidative
stress after TBI in either adults or infants
and children. Two large clinical trials in
TBI using antioxidants (polyethylene gly-
col, covalently linked to superoxide dis-
mutase and tirilazad) showed largely nega-
tive results (55, 56). Although these were
several days after severe TBI (21). Thus,
oxidative stress mechanisms might be an
attractive target for therapeutic interven-
tions in developmental central nervous
system injury.
Developmental Ramiﬁcations. Experi-
mental studies in developmental studies
of TBI support the notion that immature
brain is particularly vulnerable to oxida-
tive stress because of compromised anti-
oxidant status (60). Our prior study sup-
ported this conclusion and showed profound antioxidant depletion lasting
several days after severe TBI (21). Thus,
oxidative stress mechanisms might be an
attractive target for therapeutic interven-
tions in developmental central nervous
system injury.
Limitations of the Study. Despite the
relatively small sample size and consider-
able variability inpatient age, mechanism
of injury, and treatment, statistical sig-
niﬁcance was achieved in a multivariate
analysis between TBI patients random-
ized to hypothermia vs. normothermia
for antioxidant reserve and glutathione.
Although this study represents the larg-
est biochemical study of oxidative stress
markers in infants and children in a ran-
domized trial of hypothermia after severe
TBI, future studies with larger sample
size are needed. Despite three-fold lower

29). However, classic studies in cerebral
ischemia failed to show an effect of mild–
moderate hypothermia on energy metab-
olite levels (30).
Mild–moderate hypothermia reduces
the increase in intracellular calcium lev-
els, which is linked to excitotoxicity and
oxidative stress, after experimental cere-
bral ischemia and TBI (31–33). The ma-
majority of the free radicals that are pro-
duced after brain injury are generated by
enzymatically catalyzed mechanisms,
such as nitric oxide synthase, and by de-
regulated electron transporters in mito-
ochondria (34–38). Therefore, we specu-
late that beneﬁcial effects of hypothermia
on oxidative stress after TBI might be
explained by prevention of mitochondrial
failure and reduction in excitotoxicity.
Moderate hypothermia has been shown to
attenuate increases in CSF acetylcholine
levels (39) and brain interstitial levels of
 glutamate and aspartate seen after fluid
percussion injury (22). However, the latter
finding was not consistently observed
across experimental TBI models (40, 41).
Moderate hypothermia has also been shown
to attenuate increases in CSF gluta-
mate levels in adult severe TBI victims (23).
TBI-induced oxidative stress is impor-
tantly linked to inﬂammatory response.
Several components of the local inﬂam-
atory response to cerebral contusion are
do also favorably affected by therapeutic
hypothermia in experimental TBI, as evi-
denced by reductions in neutrophil ac-
cumulation (42–44), interleukin-1 mes-
senger RNA up-regulation (45, 46), and
inducible nitric oxide synthase activity
(47). Increases in cytokines after severe
TBI, however, are not consistently atten-
uated by therapeutic hypothermia in
adults (23, 48, 49). Similarly, macro-
phage accumulation as assessed by CSF
quino!nic acid levels was not attenuated
by moderate hypothermia in adult severe
TBI, future studies with larger sample
size are needed. Despite three-fold lower

Figure 4. Effect of hypothermia and time on cerebrospinal fluid F2-isoprostane levels after traumatic
brain injury.

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value of F2-isoprostane in patients random-
domized to hypothermia vs. normother-
mia, we were unable to show a significant attenuation of lipid peroxidation by hypo-
thermia. Sample size estimates suggest that 36 patients per group would be needed to appropriately assess for an ef-
effect of hypothermia on this parameter ac-
cepting a power of 0.8. Similarly, the small number of patients with GCS, three to four in both groups, also limits our ability to assess the effect of hypothermia in most severely injured patients. Al-
though assessment of ventricular CSF provides great promise, it reflects changes across the entire brain and not simply in the injured tissue. It is likely that biochemical alterations seen with hypothermia may, in part, be from healthy brain tissue.

Barbiturates may reduce the overall cerebral metabolism and may, therefore, change the biochemical response. Al-
though there was no statistically signifi-
cant difference between the groups for barbiturate use ($p = 0.48$), a greater number of patients in the normothermia group received barbiturates vs. hypo-
thermia group. This suggests that other ther-
apiest, such as barbiturates, might be used more frequently for intracranial pressure
control in the absence of hypothermia. In additional statistical analysis, barbiturate use did not have consistent effect on the CSF oxidative stress markers assessed in this study. Further studies with a larger sample size, including a comprehensive analysis of biochemical data as it relates to outcome, mortality, mechanism of injury (accidental trauma vs. child abuse), age, gender, other therapies (such as barbitu-
rate use and decompressive craniotomy), are needed. Assessment of the relationship between markers of oxidative stress and associated mechanisms of secondary dam-
age such as apoptosis and excitotoxicity (61, 62) could also be revealing.

Finally, although we cite these limita-
tions, we have also reported, in the same patient population, that hypothermia failed to attenuate the marked increases in CSF cytokine levels after TBI (63). Taken together, our findings mirror these observations in experimental mod-
els of TBI and support a differential effect of mild–moderate hypothermia across in-
jury mechanisms. Future research in this area might improve our understanding of mechanisms of neuroprotection by hypo-
thermia in TBI and enable us augment its beneficial effects by targeted combination therapies.

**CONCLUSIONS**

Moderate therapeutic hypothermia preserves antioxidant defenses after se-
vere TBI in infants and children. Our data support the concept that CSF represents a valuable tool for monitoring treatment effects on oxidative stress after TBI.

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