Interactive report

Hypothermia as an adjunctive treatment for severe bacterial meningitis

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Accepted 30 August 2000

Abstract

Brain injury due to bacterial meningitis results in a high mortality rate and significant neurologic sequelae in survivors. The objective of this study was to determine if the application of moderate hypothermia shortly after the administration of antibiotics would attenuate the inflammatory response and increase in intracranial pressure that occurs in meningitis. For this study we used a rabbit model of severe Group B streptococcal meningitis. The first component of this study evaluated the effects of hypothermia on blood–brain barrier function and markers of inflammation in meningitic animals. The second part of the study evaluated the effects of hypothermia on intracranial pressure, cerebral perfusion pressure and brain edema. This study demonstrates that the use of hypothermia preserves CSF/serum glucose ratio, decreases CSF protein and nitric oxide and attenuates myeloperoxidase activity in brain tissue. In the second part of this study we show a decrease in intracranial pressure, an improvement in cerebral perfusion pressure and a decrease in cerebral edema in hypothermic meningitic animals. We conclude that in the treatment of severe bacterial meningitis, the application of moderate hypothermia initiated shortly after antibiotic therapy improves short-term physiologic measures associated with brain injury. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system
Topic: Infectious diseases

Keywords: Meningitis; Hypothermia; Intracranial pressure; Cerebral perfusion pressure; Inflammation; Cleaved Tau protein

1. Introduction

Current management of brain injury emphasizes the maintenance of adequate physiologic substrate and attenuation of the inflammatory response in an attempt to prevent secondary brain injury. This approach applies the principles of improved cerebral perfusion and oxygen delivery in all patients with techniques to decrease oxygen consumption in the most severely affected [30]. Current recommendations include lowering intracranial pressure (ICP), maintaining an adequate cerebral perfusion pressure and avoiding hyperventilation [43,49]. This approach to the treatment of brain injury is developed largely from work on traumatic brain injury [48]. The study of the application of these principles in other forms of brain injury (i.e. infectious) is less well established.

Providing adequate oxygen delivery and cerebral perfusion does not reverse the primary insult and the injured brain responds with a complex inflammatory cascade that is responsible for much of the secondary damage observed. The infiltration of neutrophils [6,17,60] the production of inflammatory mediators [9,36,44] and the liberation excitatory amino acids [12,59,29] are common findings in brain injury. Additionally, meningitic brain injury results in areas of ischemia and necrosis secondary to vasculitis and areas of cerebritis secondary to neutrophil infiltration [32,57].
Cerebral edema with an increase in ICP and sudden herniation is the often fatal outcome of bacterial meningitis [26,31,46]. The raised ICP common to patients with meningitis often occurs within 12 h of admission to the hospital [27,46,47]. This time coincides with the increase in the inflammatory response generated by antibiotic therapy. This increase in the inflammatory response exacerbates blood–brain barrier dysfunction and cerebral edema [53]. Recognizing the sequence of these events offers a window of opportunity for studying interventions designed to attenuate the inflammatory response and treat or prevent secondary brain injury.

To be successful interventions designed to attenuate the inflammatory response, brain edema and increased ICP described in meningitis should modulate multiple points of the cascade of events that leads to increased brain damage. The neuroprotective role of hypothermia has been known for some time and hypothermia has been demonstrated to have a beneficial effect at multiple points of brain injury progression [40,50]. Moderate hypothermia reduces cytokine production, nitric oxide generation, leukocyte infiltration and the release of excitatory amino acids [18,22,63]. A decrease in cerebral metabolism and preservation of energy stores has been documented, as well as, a decrease in cerebral edema [8,45,62]. In our laboratory we have demonstrated that the application of moderate hypothermia decreases excitatory amino acid release and neuronal stress in meningitis [29]. Moderate hypothermia has also been utilized in traumatic brain injury to control increased intracranial pressure that has failed conventional therapy [55].

We postulate that the application of moderate hypothermia shortly after the administration of the antibiotics will attenuate the inflammatory response and increase in ICP that occurs in meningitis. Instituting hypothermia following antibiotic therapy has two characteristics that make its study appealing. Hypothermia following antibiotic therapy simulates a realistic clinical situation. The second advantage is that because inflammation increases dramatically following antibiotic administration the use of hypothermia as a therapeutic intervention will be coordinated with the time during which inflammation is increased. This study was performed in two parts. The first was to evaluate the effects of moderate hypothermia on the integrity of the blood–brain barrier and inflammatory markers of brain injury. The second was to evaluate the effects of hypothermia on intracranial pressure and the maintenance of an adequate cerebral perfusion pressure in animals with severe bacterial meningitis.

2. Material and methods

2.1. Animal preparation

This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Academy Press — revised 1996) and was performed with the approval of the Wright State University Laboratory Animal Care and Use Committee. Male white New Zealand rabbits (2.5–3 kg) were housed under conventional conditions. All surgical procedures and the administration of bacteria were performed under anesthesia. Anesthesia consisted of an intramuscular administration of ketamine (30 mg/kg) and xylazine (3 mg/kg) followed by a continuous administration of Isoflurane (1.5%) and nitrous oxide (50%).

Group B Streptococcus — type III (GBS) were cultured overnight in Todd–Hewitt broth and grown to logarithmic phase. The morning of the experiment the bacteria were washed and re-suspended in sterile saline. Meningitis was induced by inoculation into the cisterna magna of the animals with 0.4 ml of the GBS suspension after removal of 0.4 ml of cerebrospinal fluid (CSF). Sham animals received an inoculum containing sterile saline.

2.2. Randomization

Prior to randomization and sixteen hours after GBS inoculation the severity of meningitis was determined by two observers assigning a clinical severity score. Severity of illness was scored as follows: 0=Normal activity, 1=Reduced ambulation, 2=inability to stand for >5 s, 3=evidence of paralysis, 4=actively seizing for more than 5 min or comatose. Animals were paired by severity of clinical symptoms and randomized to hypothermic (32–34°C) or normothermic (37–39°C) conditions. Animals were enrolled until there was a minimum of six survivors in each treatment group. Four control (Sham) animals underwent the entire procedure but did not receive the bacterial inoculum and were handled as animals in the normothermic group.

2.3. Induction of hypothermia

Following randomization and instrumentation animals in the hypothermic group were rendered hypothermic by covering their torso with a plastic bag containing ice. Stable rectal temperature conditions (hypothermic, 32–34°C; normothermic, 37–39°C) were obtained within 1.5 h of initiating the antibiotic therapy. In a pilot study rectal and brain temperatures were equivalent in this model (data not shown). Instrumentation involved placement of a tracheotomy, a rectal probe, as well as, arterial and venous catheters. Ventilatory support was initiated and titrated to maintain a pH of >7.28, a PaCO₂ of between 30 and 40 Torr and a PaO₂>100 Torr. Following instrumentation animals were given a 20 ml/kg bolus of Hespan (Excel®, Irvine, CA) and a continuous saline infusion at 20 ml/h was started and continued for the duration of the study. Blood gasses were obtained every 2 h. If the PaCO₂ was within the target range, sodium bicarbonate was adminis-
tered at a dose of 0.5 mEq/kg or 1 mEq/kg for a pH<7.2 or pH<7.1 respectively. The temperature was monitored with a rectal probe Physitemp 701 1 HT (Physitemp Instrument Inc., Clifton, NJ). The temperature was maintained in both groups with warming blankets Baxter K-MOD 100 (Baxter Health Corporation, Deerfield, IL).

2.4. Protocol — the effects of hypothermia on inflammation

In this component of the study animals received 0.4 ml of GBS (10^3 cfu/ml). Sixteen hours after inoculation the animals received ceftriaxone (Roche Laboratories) (50 mg/kg IV), were instrumented and maintained under temperature control conditions for 10 h. A dopamine infusion was initiated and titrated to maintain a mean arterial blood pressure of greater than 40 mmHg in all animals. The hypothermic animals were re-warmed prior to euthanasia. At the conclusion of the study anesthesia was briefly lightened to observe the presence of corneal reflexes and spontaneous respirations. The study animals then underwent a second cisternal tap for CSF collection and blood samples were obtained by cardiac puncture. The animals were euthanized by an intracardiac administration of a saturated solution of KCl.

Following euthanasia an autopsy was immediately performed. The cranial vault was opened and its contents extracted en masse. Following removal the right brain was isolated, stripped of the meninges, dissected on filter paper and frozen. In three animals from each group coronal sections of the left brain were obtained fixed in formaldehyde, embedded in paraffin and cut for the preparation of microscopic slides. The slides were stained with hematoxylin and eosine according to standard techniques.

2.5. Measurements of blood–CSF barrier function and Infection

Blood and CSF specimens were centrifuged, the supernatant collected and rapidly frozen for further analysis. Alteration of blood–CSF barrier function was evaluated by measurement of protein and glucose content in CSF and serum samples. Protein content was measured spectrophotometrically utilizing the Bradford method (Bio Rad Sigma Diagnostics®). Glucose was measured spectrophotometrically utilizing a commercially available assay (Glucose HK, Sigma Diagnostics®).

Bacterial presence was evaluated by culturing 10 μl of both CSF and blood on 5% blood agar plate at 37°C, followed by colony counts 24 h later.

2.6. Measurement of NO production

Nitric oxide was determined by measuring nitrate and nitrite concentrations, the stable degradation products of NO, in CSF and serum samples [67]. Nitrite was determined after enzymatic reduction of nitrate to nitrite using nitrate reductase (670 mU/ml) with NADPH (160 μM) at ambient temperature for 3 h. Following incubation an equal volume of the Griess reagent (1% sulfanilamide and 0.1% naphthylethlenediamide in 5% phosphoric acid) was added and nitrite concentrations were measured spectrophotometrically at 550 nm.

2.7. Measurement of myeloperoxidase activity

Myeloperoxidase activity (MPO) in brain tissue was determined as previously described with minor modification [6]. The mid and anterior portions of the left hemisphere were homogenized in a solution containing 50 mM Tris–HCl (pH 7.4) at a ratio of 4 ml/g of wet weight. An aliquot of 1 ml of the homogenate was added to 20 ml of 5 mM phosphate buffer (pH 6) and centrifuged twice at 4°C. The supernatant was discarded, the pellet washed in phosphate buffer and re-suspended in 0.5% hexa-decyl-trimethyl-ammonium bromide. Three cycles of freezing/thawing in liquid nitrogen followed by sonication were performed before incubation of the sample for 20 min at 4°C. An aliquot of the supernatant was mixed with a solution of tetramethyl benzidine 3.9 mg/ml in di-methyl formamide and 0.004% of H2O2. The rate of change in absorbance was measured spectrophotometrically at 405 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol of hydrogen peroxide per minute at 25°C and expressed as units per gram of protein. The protein content of the homogenized brain was measured spectrophotometrically by the Bradford method (Bio Rad Sigma Diagnostics®).

2.8. Protocol — the effects of hypothermia on cerebral perfusion and edema

We increased the bacterial load to 5×10^5 cfu/ml and the dose of ceftriaxone to 100 mg/kg in this component of the study. Following the administration of antibiotics and instrumentation animals in the hypothermic group were cooled as previously described. Animals were instrumented as previously described with the addition of an intracranial pressure monitor (Camino®, San Diego, CA) being placed by standard technique. After the surgical procedures were performed nitrous oxide was discontinued and the isoflurane was decreased to 0.5%. An infusion of diazepam (0.7 mg/kg/h) and fentanyl (35 μg/kg/h) was administered during the remainder of the study. Animals were maintained hypothermic for 6 h.

2.9. Hemodynamic management

Maneuvers to maintain mean arterial pressure above 45 mmHg were as follows. If the mean arterial pressure fell a second bolus of 20 ml of Hespan (Excel®, Irvine, CA) was given and repeated once more if the blood pressure did not
improve. If volume resuscitation was inadequate a continuous dopamine infusion was initiated and titrated to maintain an arterial blood pressure greater than 45 mmHg. During the last hours of the protocol the normal saline infusion was adjusted so that all animals received the same water load. Before euthanasia, the hypothermic animals were re-warmed and the anesthesia was briefly lightened to observe the presence of corneal and paw reflexes as well as spontaneous respiration. Bacterial presence was evaluated in this group also by cultivating 10 μl of both CSF and blood on 5% blood agar plate at 37°C, followed by colony count 24 h later.

Cerebral edema was determined by comparison of the brain wet to dry ratio. Briefly, the brain was removed, placed on filter paper and dried in a vacuum oven at 105°C. Drying continued until a stable dry brain weight was obtained. Brain water content is expressed as water per 100 g dry weight.

2.10. Measurements cleaved Tau protein (cTau)

A post-hoc analysis of available CSF samples was performed to detect the presence of cTau in CSF. Seven pre-meningitic samples and nine from the end of the study period were available (2 Sham, 4 normothermic, 3 hypothermic) were available. Measurement of CSF cTau employs a previously characterized ELISA assay [65]. This ELISA employs three Mabs (7A5, 8A12, and 12B2) that recognize forms of cleaved MAP-tau present in human CSF. Immulon 2 plates are coated with affinity-purified Mab 12B2 (100 μl/well, 5 mg/ml) for 1 h and then coated overnight with 5% nonfat dry milk and 0.5% gelatin in Tris buffered saline (TBS). Plates are washed with 0.1% Tween in TBS (TBST), and serial dilutions of rabbit CSF added in triplicate, incubated for 1 h, and then washed with TBST. One hundred μl/well of HRP-conjugated 7A5 and 8A12 diluted 1:2000 is added for 1 h, plates washed with TBST and 100 μl/well of a 3 μg/ml solution of biotin-tyramine in 50 mM Tris–HCl, 0.001% H2O2 (pH 8.0) added for 15 min. Color is developed with Vector ABC-AP when compared to Shams (hypothermic 39°C) and 100 μl of both CSF and blood on 5% blood agar plate at 37°C, followed by colony count 24 h later.

Cerebral edema was determined by comparison of the brain wet to dry ratio. Briefly, the brain was removed, placed on filter paper and dried in a vacuum oven at 105°C. Drying continued until a stable dry brain weight was obtained. Brain water content is expressed as water per 100 g dry weight.

2.11. Data analysis

All data are presented as mean±standard error for each experiment. Differences between experimental groups were determined by unpaired t-test with the Bonferroni correction for multiple comparisons. Regression analysis was utilized when the association between continuous variables was sought. Determination of changes in heart rate, blood pressure, intracranial pressure and cerebral perfusion pressure between experimental groups utilized repeated measures factorial ANOVA. Mann–Whitney U test was utilized for no-parametric data. Results were considered significant at P<0.05

3. Results

3.1. Hypothermia attenuates markers of inflammation

In the initial experiment 33 animals were used. Eight animals died during the instrumentation process and were excluded from analysis. The experiment was carried out on the surviving rabbits: eleven infected animals were in the hypothermic group, ten infected animals were in the normothermic group and four animals were used as the control (Sham) group. All animals receiving the intrathecal injection of GBS animals developed clinical signs of meningitis characterized by meningismus, lethargy, or inability to ambulate within 16 h. The average clinical severity score at entry to the study was 1.4±0.2 in the normothermic animals and 1.5±0.2 in the hypothermic animals (p=NS). All sham animals were behaving normally upon entry into the study.

3.2. The effect of hypothermia on glucose ratio, CSF protein and NO production

Analysis of the CSF of normothermic animals demonstrated the characteristic increase in CSF protein and a decrease in the CSF/serum glucose ratio that is the hallmark of bacterial meningitis (Table 1) CSF protein in the hypothermic animals was significantly decreased when compared to normothermic animals, but remained elevated when compared to Shams (hypothermic 39±5 mg/dl vs. normothermic 56±6 mg/dl; P<0.05). Evaluation of the CSF/serum glucose ratio revealed a decrease in the glucose ratio in normothermic meningitic animals with

<table>
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<tr>
<th>Variable</th>
<th>Sham</th>
<th>Normothermia</th>
<th>Hypothermia</th>
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<tbody>
<tr>
<td>Glucose ratio (%)</td>
<td>55±7</td>
<td>39±4*</td>
<td>56±7*</td>
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<tr>
<td>CSF protein (mg/dl)</td>
<td>6±2</td>
<td>56±6*</td>
<td>39±5*</td>
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<td>CSF nitric oxide (mmol/l)</td>
<td>26±2</td>
<td>46±5*</td>
<td>31±5*</td>
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<td>Myeloperoxidase (U/gram)</td>
<td>7.2±0.9</td>
<td>19.3±2.9*</td>
<td>12.7±1.4*</td>
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# Values are equal to the mean (±S.E.M.). CSF; cerebral spinal fluid. Glucose ratio is equal to the serum glucose/CSF glucose*100. *P<0.05 when comparing normothermic animals to hypothermic animals. Significance determined by t-test with Bonferroni procedure for multiple comparisons.
preservation of the normal glucose ratio in the hypothermic group. (hypothermic 56±7% vs. normothermic 39±4%; P<0.05).

The presence of nitric oxide was determined as a marker of CNS inflammation. Nitric oxide was determined by analysis of serum and CSF nitrate/nitrite levels as previously described. Serum nitrate/nitrite values were similar between sham and infected animals. (Sham 249±18 μmol/l vs. normothermic 289±81 μmol/l vs. hypothermic 213±28, p=NS). Differences in serum nitrate/nitrite levels in the hypothermic and normothermic groups did not reach statistical significance. However, meningitis induces an increase in nitric oxide levels in the CSF of infected animals (Shams 26±2 μmol/l vs. normothermic 46±5 μmol/l; P<0.05). Meningitic animals in the hypothermic group had CSF nitrate/nitrite levels that were significantly lower than their normothermic counterparts (hypothermic 31±5 μmol/l vs. normothermic 46±5 μmol/l, P<0.05) (Table 1). There was a correlation between the increase in CSF nitric oxide and decreased glucose ratio (r²=0.259, P<0.05), between nitric oxide levels and increased CSF protein (r²=0.241, P<0.05) and between CSF nitric oxide and myeloperoxidase activity (r²=0.254, P<0.05).

3.3. Neutrophil infiltration

Histologic examination of the brain of animals with meningitis using hematoxylin and eosin staining revealed extensive neutrophil exudate in the subarachnoid space with a patchy pattern of neutrophils infiltrating the cortical surface. Fig. 1 depicts a representative profile of inflammation in infected animals. These lesions were distributed in a heterogeneous pattern in both normothermic and hypothermic animals. Because of the heterogeneous pattern we could not find an acceptable method for quantifying differences between the two groups.

Evaluation of myeloperoxidase in brain tissue of experimental animals was determined as a marker of neutrophil infiltration. Analysis demonstrated an increase in tissue MPO activity in the brain of infected animals compared to shams (Table 1). When the brain tissue was evaluated, we noted a significant reduction in tissue MPO in the hypothermic group when compared to normothermic animals (hypothermic 12.7±1.4 U/gram vs. normothermic 19.3±2.9 U/gram; P<0.05).

3.4. Bacterial clearance

CSF cultures obtained at the end of study demonstrated bacterial growth in 19 of 21 animals. One animal from each of the study groups did not have bacterial growth. Colony morphology and gram stain was consistent with GBS. Quantitative bacterial evaluation was not statistically different between groups (hypothermic 3.1±0.3 vs. normothermic 2.9±0.3 log CFU/ml; p=NS). Blood cultures were negative for all animals in both groups. There was no correlation between bacterial presence and any of the above measured parameters.

3.5. Hypothermia attenuates increased intracranial pressure

Following an intrathecal injection of GBS all animals developed clinical signs of meningitis characterized by meningismus, lethargy or an inability to ambulate. Eleven animals died from meningitis prior to randomization and were excluded. Following instrumentation and randomization, 9 animals in the normothermic group and 8 animals in the hypothermic group were entered into the study. The average clinical severity score at entry to the study was 2.2±0.3 in the normothermic animals and 2.8±0.4 in the hypothermic animals (p=NS). Three deaths occurred in the normothermic group (3 of 9) and one in the hypothermic group (1 of 8) after randomization but prior to completion of the study. Animals that died demonstrated an increased intracranial pressure, fixed dilation of the pupil, white out of the retinal vessel in the presence of cardiac function (brain herniation). In this group of animals bacterial cultures of the blood were negative in all animals. Cultures of the CSF were not performed because...
of the possibility of contamination resulting from placement of the ICP monitor.

3.6. ICP and CPP

All infected animals demonstrated an increase in ICP and decreased blood pressure when compared with the Sham group. Prior to cooling, animals in the hypothermic and normothermic groups demonstrated no difference in ICP, CPP, mean arterial pressure or heart rate. As can be seen in Fig. 2a there is a significant reduction of the ICP in the hypothermic group ($P<0.05$). This reduction remains throughout the experiment though this decrease in ICP does not fall to the level of the non-meningitic (Sham) animals. Cerebral perfusion pressure is affected while mean arterial pressure was maintained without a significant difference between the two groups by titrating the dopamine infusion. (Fig. 2b,c) Heart rate in the normothermic animals was greater than either the hypothermic or sham treated animals ($P<0.05$).

During this part of the study it was noted that hypothermic animals exhibited a rapid decrease in ICP in the first 2 h after initiating external cooling. Because this largely occurred during instrumentation two additional animals were instrumented prior to external cooling. These animals were cooled after instrumentation about 18 h after inducing meningitis to explore the relationship between the induction of hypothermia and intracranial pressure. As can be seen in Fig. 3, the ICP remains elevated until the core temperature decreases below 36°C. Below this temperature ICP readily decreases with this decrease being seen at about 90 min after initiating cooling.

3.7. Cerebral edema

To evaluate if the decreased ICP observed in hypothermic animals had a relationship with the development of cerebral edema we measured the brain water content in each group. Hypothermia induced a small but statistically significant difference in brain water content between the two groups. The average brain water content of normothermic meningitic animals was $489\pm13$ gH2O/100 g dry weight and that of hypothermic meningitic animals was $449\pm8$ gH2O/100 g dry weight ($P<0.05$). The brain water content of the Sham animals was intermediate to these values and was $461\pm7$ gH2O/100 g dry weight and the difference was not significant when compared to either the hypothermic or normothermic groups.

3.8. Cleaved Tau protein in CSF

cTau was undetectable in the CSF in the pre-meningitic samples and after the experiment in the Sham operated animals. In contrast cTau was elevated in all animals after meningitis (normothermic, $28.7\pm14$ hypothermic $29.9\pm5$ ng/ml).
Evidence supporting the use of hypothermia to attenuate brain injury following trauma dates back greater than 40 years [50]. Hypothermia improves neurologic outcome in animal models and human clinical trials of traumatic brain injury [14,40]. Acceptance of hypothermia as a treatment modality has been limited by the acknowledgment that hypothermia is known to cause myocardial depression, arrhythmias, coagulopathy and immune dysfunction. The adverse effects of hypothermia are obviated by the use of moderate hypothermia and a slower rather than rapid rate of cooling [39]. Moderate hypothermia has been generally defined as a temperature greater than 30°C, with most studies targeting a temperature of 32–34°C.

Moderate hypothermia applied after an insult plays a role in protecting neurons in ischemic and traumatic brain damage by modifying multiple points of the cascade of events that leads to secondary injury [18,22]. Hypothermia preserves cellular energy stores reducing the cerebral metabolic rate for oxygen associated with electrophysiological activity and homeostatic functions required to maintain cellular integrity [8,45,16]. It is reported that cerebral metabolic rate decreases approximately 7% for each degree Celsius reduction in temperature [8]. Further cerebral protective effects include the preservation of the blood–brain barrier function, a decreased neutrophil infiltrate and a reduction in the release of excitatory amino acids and cerebral edema [15,56,63]. These beneficial effects appear to be secondary to a suppression of the inflammatory cascade and not due to a delay in the injury process [41].

Many of the pathophysiologic processes present in traumatic and ischemic brain injury are present during meningitis [7,19,20]. Breakdown in the blood–CSF barrier, leukocyte accumulation, an increase in nitric oxide and secondary areas of ischemia and necrosis are well characterized during meningitis [32,35,57]. Our previous work has demonstrated an attenuation of excitotoxic amino acid release and decreased neuronal stress with the application of moderate hypothermia in bacterial meningitis [29].

The goal of this study was to determine if the salutary effects of hypothermia could be extended to ameliorate the secondary brain injury associated with meningitis. Our study demonstrated that the application of moderate hypothermia in a model of severe bacterial meningitis preserves markers of blood–brain barrier function, decreases CSF nitric oxide, and decreases myeloperoxidase activity in brain tissue. We have further demonstrated that hypothermia decreases intracranial pressure without negatively effecting blood pressure thereby allowing for maintenance of an improved cerebral perfusion pressure. The efficacy of ensuring an adequate perfusion pressure has recently been demonstrated in adults with bacterial meningitis [42]. Our study has also documented a small but statistically significant decrease in brain water in the hypothermic animals suggesting a decrease in cerebral edema even in this short-term study. The increase in the CSF nitrate/nitrite in meningitis with no significant change in serum nitrate/nitrite has been reported as evidence of in-situ NO production and has been correlated with increased morbidity [1,11,13,33,34,36]. In line with these findings, we found that CSF nitrate/nitrite levels are increased in meningitis and this increase is attenuated by hypothermia.

Neutrophil infiltration contributes to tissue damage by releasing radicals and oxidant molecules [37,61]. A recent study showed that hypothermia decreases the influx of leukocytes into the CSF of animals with meningitis [2]. In this study we sought to evaluate neutrophil infiltration by histology and determination of myeloperoxidase activity. Because of the heterogenous distribution of neutrophils an acceptable method of quantifying them was not found. Myeloperoxidase activity has been used as a marker of neutrophil presence and has been evaluated in different types of brain injuries [7,25,28]. Although the MPO gene is expressed in the myeloid lineage, MPO protein remains stored in large quantities in neutrophils in the azurophilic granules [23]. We have documented a decrease in MPO activity in the hypothermic group. We interpret this difference to be secondary to a decrease in neutrophil accumulation. Supporting this interpretation, we recently characterized integrin expression on CSF neutrophils in meningitis and have observed that the activation of β1-integrins on extravasated neutrophils is decreased by hypothermia [51,52]. β1-integrins are adhesion molecules involved in the binding of neutrophils to the extracellular matrix [10]. The decrease in integrin activation may be in part responsible for the decrease in neutrophil infiltration.

In our study the beneficial effects of hypothermia do not appear to be secondary to changes in the degree of...
infection. However, due to our short observation period, we cannot rule out the possibility that hypothermia may adversely affect bacterial clearance from the subarachnoid space. In this regard, it is crucial to note that delayed sterilization of the CSF in meningitis has been associated with an adverse outcome [38]. Hypothermia also has been found to interfere with the up-regulation interleukin-1β after traumatic brain injury [21,24]. Interleukin-1β mediates the increase in nerve growth factor, an endogenous neuroprotective factor. Thus, raising the possibility that by suppressing the inflammatory cascade may also hinder the reparative process [21].

Tau proteins are structural microtubule binding proteins primarily localized in the axonal compartment of neurons. Functionally, Tau binds to axonal microtubules resulting in the formation of axonal microtubule bundles. Loss of axonal microtubules resulting from direct or indirect injury to central nervous system axons is a common feature of several types of brain injury [64,66]. This loss of axonal microtubules following injury releases intracellular microtubule binding proteins, such as Tau, into the extracellular space, where they are transported to the CSF. CSF levels of a cTau reflect axonal damage after traumatic head injury [65]. We found that cTau protein is elevated in CSF during meningitis. There is no previous data that allows any speculation on the relationship between the time or severity of meningitis and the appearance of cTau. The post-hoc nature of this part of the study only allow us to conclude that cTau is elevated in meningitis and further studies are necessary to see if it could be a utilized as a biological marker of neurological injury in meningitis. Another limitation is that the monoclonal antibodies were developed against human protein. Based on our results we feel that sufficient cross reactivity exists to support our conclusion.

In an attempt to make this study more clinically relevant the application of hypothermia occurred following the administration of antibiotics in a live-bacterial model. Because the application of hypothermia is likely to be reserved for only the most severely affected patients our model used a substantial bacterial inoculum. Further, antibiotic therapy was used to stimulate an increase in the inflammatory response and the application of hypothermia was used to coincide with the time that the inflammatory response was greatest. The results of this study suggest a beneficial short-term effect of the application of hypothermia in severe bacterial meningitis. Mortality from meningitis remains between 7 and 20% despite advances in antibiotic therapy [4,54]. Additionally, significant neurologic sequelae occur in greater than 20% of survivors [5]. At the time a patient arrives for medical attention, the conventional treatment of using antibiotics induces a massive release of bacterial products with a subsequent exacerbation of inflammation and resultant secondary brain damage [3,58]. This rapid exacerbation of the inflammatory response underscores the fact that mortality due to meningitis is usually secondary to brain herniation within the initial 12 h following admission and initiation of medical treatment [27,31,46,47].

We conclude that the initiation of moderate hypothermia at the time of antibiotic therapy in an animal model of bacterial meningitis modulates the inflammatory response. Hypothermia produces an attenuation of blood–brain barrier disruption, nitric oxide activation and decreases myeloperoxidase activity. Improvement in the increase in intracranial pressure with preservation of cerebral perfusion pressure accompanies treatment with moderate hypothermia. Further studies to investigate whether these effects are transitory and whether hypothermia ultimately affects morbidity and mortality should be entertained.

Acknowledgements

This work was supported by a grant from The Children’s Medical Center Research Foundation, Dayton OH. The author’s would also like to thank Roche Laboratories for the gift of the ceftriaxone used in this study.

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