Hypoxic-ischemic injury may cause multisystem organ damage with significant aberrations in clotting, renal, and cardiac functions. Systemic hypothermia may aggravate these medical conditions, such as bradycardia and increased clotting times, and very little safety data in neonatal hypoxic-ischemic injury is available. This study reports a multicenter, randomized, controlled pilot trial of moderate systemic hypothermia (33°C) vs normothermia (37°C) for 48 hours in infants with neonatal encephalopathy instituted within 6 hours of birth or hypoxic-ischemic event. The best outcome measures of safety were determined, comparing rates of adverse events between normothermia and hypothermia groups. A total of 32 hypothermia and 33 normothermia neonates were enrolled in seven centers. Adverse events and serious adverse effects were collected by the study team during the hospital admission, monitored by an independent study monitor, and reported to Institutional Review Boards and the Data and Safety Monitoring Committee. The following adverse events were observed significantly more commonly in the hypothermia group: more frequent bradycardia and lower heart rates during the period of hypothermia, longer dependence on pressors, higher prothrombin times, and lower platelet counts with more patients requiring plasma and platelet transfusions. Seizures as an adverse event were more common in the hypothermia group. These observed side effects of 48 hours of moderate systemic hypothermia were of mild to moderate severity and manageable with minor interventions. © 2005 by Elsevier Inc. All rights reserved.

Introduction

In the translation of hypothermia as a neuroprotective strategy from the well-established animal models to human neonates with neonatal encephalopathy, clinical trials must begin to establish the safety of the therapy. Although animal models have consistently demonstrated efficacy, they do not readily allow investigation of the side effects that may be observed in human neonates who are making a transition from intra- to extraterine life. With the inherent difficulty of studying a perinatal asphyxia model, only a few studies have investigated the safety of hypothermia in the unique setting of infant animals within 24 hours after birth [1,2]. Investigations in infant piglets with hypoxic-ischemic injury and case reports of hypothermia for human cardiac arrhythmias have suggested an increase in systemic, as well as pulmonary vascular resistance, decreased heart rate resulting in decreased cardiac output, electrolyte abnormalities, and increased risk of sepsis with hypothermia [3-6].

From clinical experience with hypothermic cardiopulmonary bypass for cardiac and neurosurgery and small pilot trials of hypothermia in neonates, we anticipated coagulopathy and bradycardia as the primary side effects of systemic hypothermia in neonatal encephalopathy.
However, these side effects may also occur in normothermic hypoxic-ischemic infants as a result of multorgan system damage alone. The present randomized, controlled pilot trial was conducted to describe in detail the adverse effects of hypothermia, compare these rates among treatment groups, and obtain rates of adverse outcomes for a power and risk benefit analysis for a larger-scale clinical trial.

**Methods**

Infants qualified for the study if they were ≥35 weeks gestation, ≥2000 gm birth weight, were ≥6 hours after birth or hypoxic-ischemic insult. Entry criteria required one clinical indication of hypoxic-ischemic injury (cord gas pH ≤ 7.0 or base deficit ≥13, initial infant gas pH < 7.1, Apgar score ≤5 at 10 minutes, continued resuscitation after 5 minutes, fetal bradycardia with heart rate < 80 beats per minute lasting ≥15 minutes, or postnatal hypoxic-ischemic event with oxygen desaturation <70% or arterial oxygen pressure <35 for 20 minutes with evidence of ischemia [chest compressions, hypotension, hemorrhage]) and two neurologic findings of neonatal encephalopathy (abnormalities of tone, reflexes, state of consciousness, seizures, posturing, autonomic dysfunction). Maternal chorioamnionitis, sepsis at birth, birth weight or head circumference <10%, or presumed chromosomal abnormality were exclusion criteria.

Randomization procedures are detailed in the accompanying article on efficacy outcomes. Briefly, infants were screened for eligibility if they had resuscitation, acidosis or low Apgar scores at birth, or an abnormal neurologic examination at 1 hour of age, and were born in or referred to a participating tertiary care center within 6 hours of birth or hypoxic-ischemic event. The parents of neonates who met entry criteria consented before randomization by web-based program to either normothermia or hypothermia treatment. Treatment was instituted initially in a participating institution’s delivery room or by their transport team at an outlying hospital. If randomized to hypothermia, ice was applied to the head and body for approximately 2 hours, then rectal temperature of 33 °C was maintained on a servo-controlled cooling blanket for 48 hours (Cincinnati Sub-Zero Blanketrol II, Cincinnati, Ohio). All patients had rectal temperature probes, indwelling central lines, pulse oximetry and cardiorespiratory monitoring, and urinary catheters. Rewarming by 0.5 °C was begun after 48 hours of hypothermia, if euveloma and normocalemia were present [12]. Morphine was used for analgesia at the attending physician’s discretion, or fentanyl if cardiovascular compromise was significant. Seizures were treated with phenobarbital, and lorazepam or phenytoin if required.

Primary outcome variables of safety were anticipated to be bradycardia and disseminated intravascular coagulopathy, and a tendency toward increased sepsis in the hypothermia vs normothermia groups.

Adverse outcome variables were initially identified as disseminated intravascular coagulopathy, cardiac arrhythmias, persistent metabolic acidosis, sepsis/pneumonia within the first 7 days of life, hypokalemia, necrotizing enterocolitis, skin injury, extension of intracranial hemorrhage, persistent pulmonary hypertension, and the need for extracorporeal membrane oxygenation.

**Laboratory Studies**

The following laboratories were obtained at times specified by the protocol: Arterial blood gases were monitored every 6 hours for 72 hours. Electrolytes, complete blood counts, and platelet counts were monitored every 12 hours for 72 hours. Coagulation studies were monitored every 12 hours until normal x 2 without blood product replacement. Liver function studies and cardiac enzymes were measured on enrollment and at 48 hours. Head ultrasounds and echocardiograms were obtained after enrollment and at 48-72 hours. Neonates were anticipated to be too unstable for transport out of the unit for other imaging studies. Electroencephalograms were obtained at 72-96 hours.

**Management of Abnormal Laboratory Values**

The management protocol for this pilot trial stipulated that pressors or volume be used to maintain mean blood pressure greater than 35 mm Hg, and blood products be used to maintain platelet counts greater than 50,000 cells/µL, prothrombin time <17 seconds, and fibrinogen >100 mg/dL. Sodium was to be maintained greater than 120 mEq/L, and potassium >3.0 mEq/L. Sodium bicarbonate was administered or ventilation adjusted to maintain pH > 7.25 and arterial carbon dioxide pressure >30 mm Hg.

**Exit Criteria**

Patients were withdrawn from treatment if they had a clinical diagnosis of sepsis or meningitis (positive blood or cerebrospinal fluid culture), or pneumonia (by chest radiograph and clinical diagnosis) in the first 36 hours of life, uncontrolled disseminated intravascular coagulopathy, or sustained bradycardia <70 beats per minute with hypotension and acidosis on inotropic medications, which did not respond to rewarming to 34°C.

**Data Collection**

Data from vital signs, neurologic examinations, medications, blood products and volume expanders, urine output, and laboratory and radiographic studies were collected over the first 5 days after enrollment for the database. Days after enrollment were not counted as calendar days, but counted as 0-23 hours after enrollment for day 1, 24-47 hours for day 2, 48-71 hours for day 3, and so forth. For example, in the hypothermia group, after 48 hours of systemic hypothermia, rewarming occurs at the beginning of day 3. Correct database entry was independently verified.

Canadian values for laboratory studies were converted from mol/L and kPa units to g/L or Eq/L and mm Hg, respectively. Canadian prothrombin times were not able to be converted, owing to different methodology, and those patients were omitted from calculations involving prothrombin times. Extracorporeal membrane oxygenation patients (four normothermia patients) were omitted from all analyses of thrombocytopenia, disseminated intravascular coagulopathy, and replacement blood products.

Dopamine and dobutamine are expressed in total amounts milligrams per kilogram of birth weight given. This measurement does not translate strictly into standard clinical expressions of micrograms/kg/minute. However, pressors are frequently titrated minute by minute, making data collection for these variables difficult. Therefore data on total amount of pressors were collected every 24 hours, which gives an accurate view of not just the lowest or highest concentrations of pressors required in a day, but the extent of cardiac support required.

**Monitoring for Adverse Events**

Abnormal laboratory/radiographic tests obtained any time during hospitalization were evaluated for clinical significance using predetermined abnormal values for expected adverse events. Adverse events are defined as conditions present any time during the hospitalization, but not present at baseline (enrollment), or a baseline condition that initially resolved and later recurred. Serious adverse events are defined as adverse events that prolonged hospitalization, were life-threatening, or required significant intervention. The designation of adverse event does not imply causation, and the possibility of a serious adverse event being related to the treatment is determined by the principal investigator at each site, according to Institutional Review Board regulations. All adverse events
and serious adverse effects during the hospital admission were collected by the study team and recorded in the case report form. The independent monitor performed 100% monitoring of data points in the case report form during site visits for the first 34 patients in the study and for the first 3 to 4 patients at each site. Thereafter, only entry criteria, laboratory values, medications, blood product replacement, adverse events, and serious adverse effects were monitored. After the initial hospitalization, adverse event data collection was limited to the serious adverse event of death. All adverse effects and serious adverse effects were monitored at the halfway point and annually during the study by a National Institutes of Health Data and Safety Monitoring Committee. Serious adverse effects were also reported to the lead institution’s and the local Institutional Review Boards.

Statistical Comparisons of Adverse Effects

We used chi-square or Fisher exact tests for categorical variables, the pooled t test for continuous variables that were normally distributed, and Satterthwaite t test for continuous variables when unequally distributed. Laboratory values are reported as means if normally distributed, specifically looking at mean highest or mean lowest values to analyze hypothermia’s potential adverse effects. Where not normally distributed, such as Apgar scores, medians were used with Kruskal-Wallis test for significance. Where abnormal laboratory or vital sign values would have been corrected by a therapeutic action, such as giving pressors to maintain a mean blood pressure, further detail was developed through subanalyses. These extensive subanalyses help evaluate if there are real differences between groups not readily determined by looking at simple mean values.

Results

Demographics

Seven participating sites enrolled 65 infants, 32 randomized to hypothermia, 33 to normothermia. Three hypothermia infants were withdrawn after randomization but before completion of hypothermia treatment because of parental request [2] and normal neurologic examination [1], and the latter does not have data available for safety analysis. Two normothermia patients were withdrawn between 24-36 hours of age as a result of sepsis at birth, an exclusion criterion, and are not included in this safety analysis, because they may have metabolic derangements confounded by their underlying disease. There were no significant differences in gestation, birth weight, sex, race, numbers of inborn vs outborn, or condition on enrollment (chest compressions, cord pH, cord base deficit, Apgar scores, or Sarnat stage) between the hypothermia and normothermia groups (Table 1). (Further details of clinical characteristics of the cohort are presented in the accompanying article on efficacy outcomes.)

Table 2 lists observed frequencies of adverse events by organ system, with statistical comparison of these adverse events between treatment groups whenever possible. Adverse events listed in Table 2 are defined as conditions present any time in the first 5 days but not present at baseline (enrollment), or as baseline conditions that initially resolved and later recurred. There were no significant differences in baseline medical conditions on enrollment for any side effect. Also included in Tables 3-5 are analyses of data broken by day to give an indication of events that occurred during hypothermia treatment and those that persisted after rewarming.

Electrolyte and Renal Dysfunction

There were no differences in metabolic derangements between groups, except in metabolic acidosis (pH < 7.25) after enrollment which was worse in the normothermic group. Renal dysfunction was equally common in both groups, with the exception of hematuria, which was significantly more common in the hypothermia group.

Cardiac Adverse Events

Bradydcardia (heart rate <80 beats per minute) was significantly more common in the hypothermia group, and the mean lowest heart rate was significantly lower throughout the period of hypothermia (days 1-3). After rewarming (days 4-5), neither the mean lowest heart rate nor bradycardia were different between groups (data not shown).

Hypothermia may have deleterious effects on other aspects of cardiac function. Significantly lower mean blood pressures were observed on day 3 in the hypothermia group (mean 37
Compared with the normothermia group (44 ± 10 mm Hg, P = 0.006). Cardiac echocardiograms obtained between 0-24 hours and repeated 48-72 hours after enrollment indicated worsening right ventricular function in two hypothermia patients. There were no cases of worsening in left ventricular function in either group. However, greater and longer cardiac inotropic support was required in the hypothermia group compared with the normothermia group (Tables 2 and 3).

Coagulopathy and Thrombocytopenia

Coagulopathy and thrombocytopenia may contribute to an increased clinical risk of bleeding. Both were more prevalent in the hypothermia group by laboratory indices, analyzed both with and without the patients on extracorporeal membrane oxygenation. More hypothermia patients required plasma and platelet transfusions than normothermia patients (Table 2).
There were usually two complete blood counts and clotting studies obtained each day on each patient, and taking the highest prothrombin time value and lowest platelet value per day yielded a truer picture of increased laboratory indices of coagulopathy in the hypothermia group compared with the normothermia group. Mean lowest platelet counts were significantly lower in the hypothermia group for every day of data collection (Table 4). The median highest prothrombin time values were significantly higher in the hypothermia group on day 4 (Table 5).

However, clinical manifestations of coagulopathy were uncommon. Central nervous system bleeds or infarcts were detected on the first head ultrasound in three hypothermia patients (old choroid plexus cyst/hemorrhage, cortical and spinal infarcts with maternal cocaine use, large thalamic and cerebellar hemorrhages with maternal blood pressure of 245/158 before delivery) and two normothermia patients (subdural hemorrhage, spinal cord infarcts). These were likely related to antenatal or intrapartum conditions, but hypothermia had been initiated for 3-12 hours before the first ultrasound. Two bleeds were detected on the second ultrasound at 48 hours after enrollment. One hypothermia patient had a rebleed of an old central nervous system hemorrhage sometime in the first 4 days of life, and one normothermia patient had a small new bleed in the corona radiata. One patient in each group had evidence of pulmonary hemorrhage, with a hemorrhage in the one normothermia patient on extracorporeal membrane oxygenation perhaps precipitated by circuit clotting and circuit change (the patient died). All were reported as serious adverse events.

**Hematologic Indices and Infections**

Hematologic indices were monitored as markers for infection. Two cases of neutropenia as an adverse event occurred in the hypothermia group, and one case of sepsis after 7 days in the hypothermia group (Table 2). However, the pilot trial numbers were too small to detect differences in such rare events as sepsis, pneumonia, or necrotizing enterocolitis.

**Pulmonary Hypertension**

Pulmonary vascular resistance has been demonstrated to be increased with hypothermia, but may also be present under normothermic conditions after hypoxic-ischemia. Therefore evaluation of both numbers and severity of this adverse event is necessary. Although pulmonary vascular resistance can not be measured directly in neonates, nitric oxide treatment and extracorporeal membrane oxygenation are surrogate measures of the severity of pulmonary hypertension. Significantly more hypothermia patients required nitric oxide than normothermia patients, but only one normothermic patient required extracorporeal membrane oxygenation as an adverse event (Table 2). The decision to go on bypass was made after randomization in that normothermic patient, and before randomization in three other normothermic patients.

### Table 3. Number of patients on pressors (excluding ECMO patients)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dobutamine Hypothermia</td>
<td>9</td>
<td>10</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normothermia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normothermia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = 0.4</td>
<td>P = 0.02*</td>
<td>P = 0.006*</td>
<td>P = 0.01*</td>
<td>P = 0.1</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Mean lowest daily platelet count (excluding ECMO patients)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothermia</td>
<td>154 ± 58</td>
<td>120 ± 56</td>
<td>111 ± 65</td>
<td>117 ± 58</td>
</tr>
<tr>
<td>Normothermia</td>
<td>202 ± 79</td>
<td>182 ± 76</td>
<td>172 ± 80</td>
<td>200 ± 81</td>
</tr>
<tr>
<td>P = 0.013*</td>
<td>P = 0.001*</td>
<td>P = 0.004*</td>
<td>P = 0.0005*</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations
ECMO = Extracorporeal membrane oxygenation

* Statistically significant P values are indicated in bold.
Other Significant Adverse Events

Seizures were significantly more common in the hypothermia group as an adverse event than in the normothermia group (Table 2). For this variable, all infants who had not seized before or during enrollment were considered to have the adverse event of seizures, which were largely clinically identified, as continuous electroencephalographic recording was not routinely performed. There was also a trend toward more abnormal electroencephalograms at 72 hours in the hypothermia group (24/26 or 92% in the hypothermia group, 18/23 or 78% in the normothermia group, \( P = 0.23 \)).

Stridor was more common in the hypothermia group, perhaps related to ventilator temperature of humidified air, which was reduced in this pilot trial from normal 36°C to 34°C. This condition may have increased the transient tracheal swelling after extubation, but stridor was quickly dispelled with inhalation of racemic epinephrine (routine treatment), and no infant required reintubation because of stridor.

Serious Adverse Events

Serious adverse events of death was also an outcome variable. The overall study death rate was 37% (24/65), with only one death occurring after hospital discharge. Using intent-to-treat analyses, 10 of 32 (31%) hypothermia infants expired, vs 14 of 33 (42%) normothermia infants, \( P = 0.35 \).

Other than death, there were five serious adverse events reported for the entire study that prolonged hospitalization, were life-threatening, or required significant intervention in the judgment of the center’s principal investigator.

Among normothermia patients, one infant had subglottic stenosis after extubation causing stridor and requiring a tracheostomy, which prolonged hospitalization. One patient had gastric bleeding after gastric tube placement for inability to feed by mouth, and required a transfusion. One patient with *Escherichia coli* sepsis was on extracorporeal membrane oxygenation when the circuit clotted, and the infant had a pulmonary hemorrhage.

Among hypothermia patients, one patient had been on dobutamine and norepinephrine for cardiac compromise at the time of enrollment, and subsequently had an acute drop in mean blood pressure to 11 mm Hg, responding to dopamine and a fluid bolus. One patient had an old choroid plexus hemorrhage documented on first head ultrasound. A subsequent head ultrasound revealed a rebleed into this same site, sometime in the first week; this was judged to be possibly related to hypothermia treatment. (This patient is included in Table 1 as extension of old bleed.)

Temperature Stability as a Safety Measure

Hypothermia patients had greater variability in temperature than normothermia patients, as detailed in the accompanying article on efficacy outcomes. The mean difference in high-low daily rectal temperatures from 24 to 48 hours after enrollment was 1.6 ± 0.6 °C in the hypothermia group vs 1.0 ± 0.5 °C in the normothermia group (\( P < 0.001 \)). Temperature variation was well tolerated in the hypothermia group, and no known adverse events were temporally related to lower temperatures. Hyperthermia, defined as temperature ≥39°C, was present in seven hypothermia patients and five normothermia patients. Hyperthermia occurred in two patients in each treatment group during the first 72 hours (includes treatment and rewarming period), and in five hypothermia patients and three normothermia patients after 72 hours. Patients who were ≥38.5°C at any time were treated with acetaminophen [4], skin probe change, bed adjustment or unswaddling [7], and hypothermia blanket [1] to maintain normal temperature range.

Discussion

Although there is clinical experience with deep hypothermia for short periods during cardiopulmonary bypass in neonates and children, there is little clinical experience with more prolonged periods of moderate hypothermia in human neonates. In an attempt to move this potential neuroprotective therapy rapidly to the clinical arena, small pilot trials were performed using either systemic or selective head cooling. Pilot trials with small numbers of patients will only reveal common, serious safety concerns and cannot address differences in these safety outcomes by treatment group. Even our larger pilot trial does not have
the power to detect infrequent adverse events or to evaluate all adverse events for statistical significance.

To help identify areas of possible increased risk with hypothermia treatment, our adverse event data were analyzed from several perspectives to provide information on the incidence and severity of these adverse events. We then looked at potential antecedents or markers for adverse events, such as absolute neutrophil count for sepsis, pressor dependence for cardiac compromise, and requirements for blood products in both treatment and control groups. This detailed analysis of observed side effects allows one to draw some conclusions about the safety of 48 hours of moderate hypothermia.

Induced hypothermia in the setting of neonatal hypoxic-ischemic injury carries an increased risk for bradycardia and mild cardiac compromise, with a longer dependence on pressors that continued after the period of rewarming. Systemic hypothermia was associated with an increase in abnormal clotting studies with higher prothrombin times and lower platelet counts for several days after rewarming, the clinical significance of which appears to be limited to greater numbers of patients receiving replacement blood products. In addition, although temperature regulation for the hypothermia group manifested larger variation than the normothermia group, and most of the hypothermia group had temperatures lower than the predefined target range at some point in treatment, no bleeding, bradycardia, or other adverse event could be attributed to these temperature variations in the pilot data.

Clinical seizures were unexpectedly more common and much later in onset in the hypothermia group compared with the normothermia group, as were electroencephalographic abnormalities at 72 hours after insult. The exact timing of hypoxic-ischemic injury is frequently unknown in neonatal encephalopathy, making data on the timing of seizures difficult to evaluate in pilot trials owing to small patient numbers. Although a delay of seizure activity might be an expected outcome of hypothermia, these data need to be further evaluated in the design of a larger trial, which should consider including continuous electroencephalographic recordings to obtain electrical correlation for clinical seizures.

Not all statistically significant adverse events observed with moderate hypothermia are clinically significant. The laboratory values for coagulopathy are good examples where there are statistical differences that may not infer clinical risk, once values are within the normal range. However, in some cases in the present study, abnormalities persisted up to days 4 and 5, after which point detailed, day-specific data for these indices were not collected. Longer periods of monitoring some of these indices and particular attention to increased severity instead of simple incidence, would be helpful to assess the safety and continued adverse effects of hypothermia.

Detection of clinical side effects with hypothermia that extend beyond the treatment period in this pilot trial should augment management of these adverse effects in a larger trial. In general, these observed side effects of 48 hours of moderate systemic hypothermia of 33°C were of mild to moderate severity and manageable with minor interventions. Continued reporting on the adverse effects of hypothermia in future trials will continue to deepen our understanding of the effects of this promising therapy.

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References