Systemic effect of prolonged moderate systemic hypothermia in neonatal hypoxic ischemic encephalopathy

PhD theses

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1. Induction

Neonatal hypoxic-ischemic encephalopathy (HIE) is an important cause of death and neurodevelopmental delay worldwide. Treatment for infants with hypoxic-ischemic encephalopathy was limited to supportive care for a long time, but efforts have been made to develop effective therapies. However, through several experimental therapies for hypoxic ischemic encephalopathy seemed promising none proved consistently successful in clinical studies. Despite the improvement in neonatal care in the last decades, the incident of neurological disabilities related to perinatal brain injury remained unchanged affecting 2-3 in 1000 newborns. However accurate Hungarian national statistical data are not available, the estimated number of infants born with HIE is 150-200 in a year.

Over the past two decades, experimental and clinical evidence has accumulated that a 3-4°C reduction of body temperature maintained for at least 72 hours in newborns with hypoxic-ischemic encephalopathy may reduce cerebral injury and improve neurological outcomes. Recent clinical trials have demonstrated that prolonged cooling of either the head or the whole body of neonates with HIE is safe and associated with reduced short-term mortality and morbidity at 18 months of age.

2. Aims

Our aim was to perform several observational studies on our study group while participating in the international, randomized TOBY trial. We felt that this trial was one of the last opportunities to do observations comparing a randomized hypothermic and normothermic study group.

There are several questions not fully investigated in hypothermia. Our plan was to collect more information during prolonged systemic hypothermia about markers of multiorgan failure and brain injury, cytokine responses, and changes in morphine metabolism before hypothermia introduced as standard care. For this purpose additionally to the TOBY protocol we collected serial blood samples at fixed time points in term infants treated with hypoxic-ischemic encephalopathy.

Our aim was to find answers for four hypotheses:

1. We hypothesized that as the main pathogenic processes of brain injury and the dysfunction of other organs are partly similar after asphyxia, hypothermia believed to attenuate hypoxic cerebral injury could also protect organs other than brain exposed to hypoxia. There are just few data concerning this issue. Therefore we investigated the effect of hypothermia on some laboratory parameters reflecting cellular necrosis and organ dysfunction characteristic for the failure of internal organs in asphyxiated neonates.

2. Secondly we wanted to evaluate the effect of systemic moderate hypothermia on levels of serum S100B and NSE proteins, their time course, and association with the aEEG and neurodevelopmental outcome as no data was available before.

3. One of the mechanisms via hypothermia believed to act is the reduction of the inflammation. The aim of our next substudy was to explore the influence of therapeutic hypothermia on serum cytokine and cortisol concentrations.

4. During our pilot study before the TOBY trial we observed severe late hypotension in some infants treated with hypothermia without clear cardiovascular cause. Discussing this we found that we use morphine treatment more often and in higher dose than other study groups, and the late cardiovascular instability can be related to morphine toxicity. Our hypothesis was that morphine pharmacokinetics are altered during prolonged moderate systemic hypothermia in asphyxiated neonates, resulting in excessively high morphine concentrations compared with infants kept at normothermia.

3. Methods

Between January 2005 and December 2007, 64 term infants were admitted to the regional level 3 neonatal care unit at the First Department of Pediatrics, Semmelweis University with the diagnosis of hypoxic-ischemic encephalopathy. These infants were screened for evidence of encephalopathy according to a 3-step eligibility system based on clinical and neurologic criteria as used in the TOBY Study. Infants were enrolled within 6 hours of birth if each of the following criteria was fulfilled. (1) infants were ≥36 weeks' gestation with ≥1 of the following: (a) Apgar score of ≤5 at 10 minutes after birth; (b) continued need for resuscitation, including endotracheal or mask ventilation, at 10 minutes after birth; (c) acidosis defined as pH ≤7.0 and/or base deficit ≥16 mmol/L in umbilical cord blood sample or any blood sample within 60 minutes of birth (arterial or venous blood); (2) moderate to- severe encephalopathy consisted of altered state of consciousness (irritability, lethargy, stupor, or coma) and ≥1 of the following: (a) hypotonia, (b) abnormal reflexes including oculomotor or pupillary abnormalities, (c) an absent or weak suck, or (d) clinical seizures; and (3) ≥30 minutes duration of amplitude-integrated electroencephalogram recording showed moderately abnormal or suppressed background amplitude-integrated electroencephalogram activity or seizures. Exclusion criteria from the TOBY were prematurity, congenital malformations, suspected metabolic disorders, absence of parental consent and age of more than six postnatal hours.

From the 64 admitted infants 24 with HIE were enrolled into the multinational, randomized and prospective TOBY Study (Total Body Hypothermia for the Treatment of Perinatal Asphyxial Encephalopathy, ISRCTN 89547571). The study was approved by the national Ethical Committee for Medical Research (591/KO/2004). 40 infants were not enrolled to the study because of the following reasons: no evidence or mild encephalopathy 22/40, unstable infants with severe HIE 9/40, admission after 5 hours of life 2/40, congenital abnormality 1/40 (diaphragmal hernia), parental consent not given 5/40, lack of human resources 1/40.

Before treatment, each patient's parents provided informed consent to participate in this study.

Infants allocated to treatment with standard intensive care and hypothermia were cooled to an aim rectal temperature of 33.5°C for 72 hours, called the hypothermia group ((HT) $n = 13$). Infants allocated to the control group (normothermia (NT); $n = 11$) were treated with standard intensive care on normothermia (37°C).

Both groups were treated with the same regimen, except for the hypothermia. All of the infants were treated with a continuous infusion of morphine-hydrochloride, with the rate adjusted according to clinical status.

After randomization, aEEG monitoring was continued and the background activity was assessed at the time points of blood sampling. Seizures were controlled with phenobarbital in both groups. If the infant remained agitated or seizures persisted, a single dose of midazolam was administered. The daily cumulative doses of morphine and other drugs were recorded. Cardiovascular instability was maintained as unit protocol. Blood and outer ear swab cultures were obtained at admission from all infants and bacterial infection was excluded. All infants received a regular antibiotic regimen *i.e.* ampicillin and amikacin during the study period.

Venous blood samples were taken at 6, 24, 48 and 72 h after birth for laboratory measurements of full blood count, electrolytes, liver function (ASAT, ALAT), lactate dehydrogenase, creatine kinase, creatinine, uric acid, coagulation and for further investigations (S100, NSE, cytokine and morphine measurements). Samples were collected via venous umbilical catheter. Blood was centrifuged; sera were separated and stored at -80°C until further measurements. Laboratory measurements of 21 infants were analysed by the commercially available tests on a Roche Hitachi 912 system Hitachi 714 automated system. S100B and NSE were measured by enzyme-linked immunosorbent assay (Roche) from frozen sample (100 µl sera) according to the manufactures instructions. 12 cytokines and vasoactive agents (interleukin (IL)-1-α, IL-1-β, IL-2, IL-4, IL-6, IL-8, IL-10, MCP-1, EGF, VEGF, IFN-γ, TNF-α), were measured from frozen sample (100 µl sera) with the Randox Cytokine and Growth factors array (Randox Lifesciences, Crumlin, United Kingdom). Serum morphine concentrations were determined with an enzymelinked immunosorbent assay (Opiates Reagent Pack, Abbott Diagnostics, Abbott Park, IL) from the frozen samples.
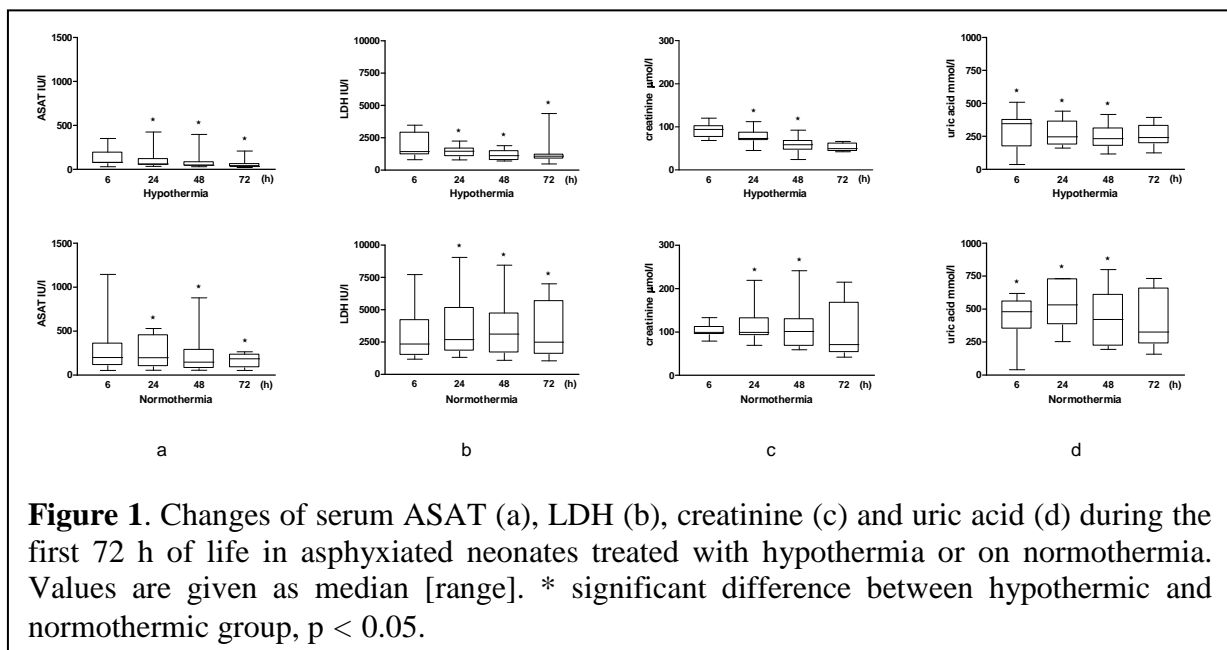
Cranial ultrasound scan was performed daily during the investigation period and reported by radiologist consultants. Brain MRI was performed between day 5 and 14 on a Philips 3T scanner obtaining T1 weighted, T2 weighted, and diffusion weighted images. Neurodevelopmental assessment (Bayley Scales of Infant and Toddler Development TM III) was performed between 18-22 months according to TOBY study protocol. Infants were classified into two groups depending on the results of the follow up examination: Survival without severe disability; and severe developmental delay (MDI and PDI <70) or death.

The analysis of the data usually was performed by the statistical software Statistica 8.0 (StatSoft Inc, Tulsa, USA). Anthropometric and clinical parameters were compared with Mann-Whitney and Fischer tests. The level of significance was set at $p < 0.05$.
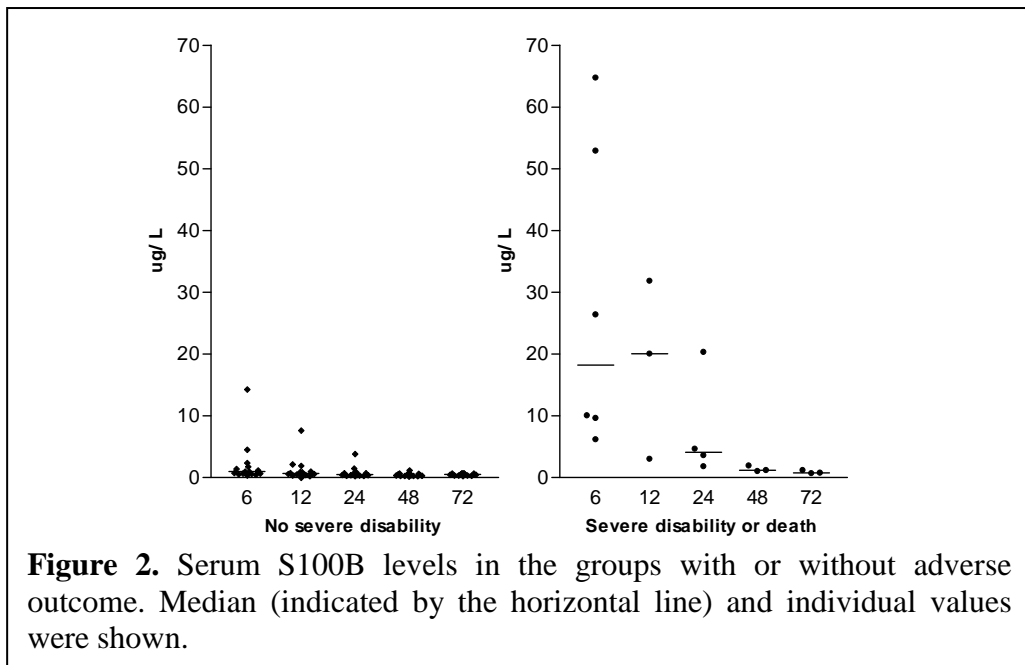
4. Results

The clinical characteristics of the infants in the HT and NT groups were similar. The time of admission was (median [range]) 1.8 [0.8-4.4] hours of age in the HT and 1.3 [1.0-4.5] hours in NT groups. The time of randomization was 3.3 (2.5-5.3) hours of age in the HT and 3.1 (2.2-5.5) hours in NT groups. Clinical parameters used to assess the severity of HIE (Apgar scores, pH base excess and lactate within the first hour of life, Sarnat scores) showed no significant difference between the two groups.

Area under curve values of aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), uric acid and creatinine during the investigated period and alanine aminotransferase (ALAT) value at 72 h were lower in neonates on hypothermia than in those on normothermia (Figure 1.). Renal failure and liver impairment affected less hypothermic than normothermic neonates (3/12 vs. 7/9, p = 0.03, 3/12 vs. 6/9 p = 0.08, respectively). Four of the 12

Figure 1. Changes of serum ASAT (a), LDH (b), creatinine (c) and uric acid (d) during the first 72 h of life in asphyxiated neonates treated with hypothermia or on normothermia. Values are given as median [range]. * significant difference between hypothermic and normothermic group, p < 0.05.
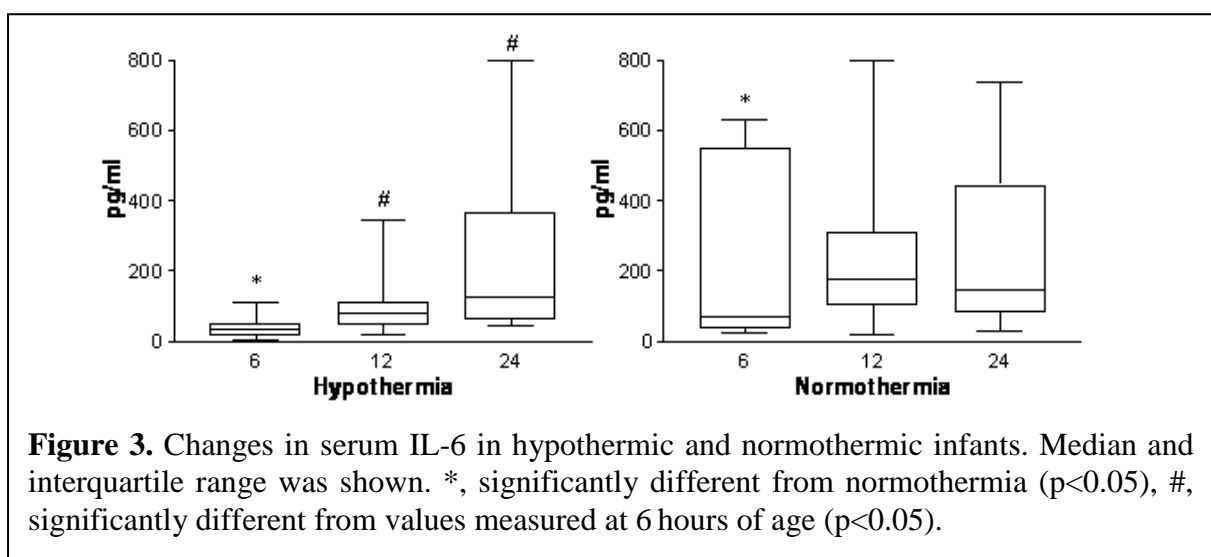
hypothermic and 6 of the 9 normothermic neonates developed multiorgan failure.

Serum S100B and NSE levels were grossly elevated in both hypothermic and normothermic groups. Compared to values at 6 hours of age, S100B values decreased over time in both groups (NT: p=0.002, HT: p=0.04). Serum S100B values were lower in hypothermic infants compared to normothermic infants (p=0.047 at 48 hours). Serum S100B (Figure 2.) and NSE values were significantly higher in infants who died or developed severe neurological impairment (S100B, p<0.05 at all time points; NSE, p=0.036 at 24 hours of age).

Figure 2. Serum S100B levels in the groups with or without adverse outcome. Median (indicated by the horizontal line) and individual values were shown.
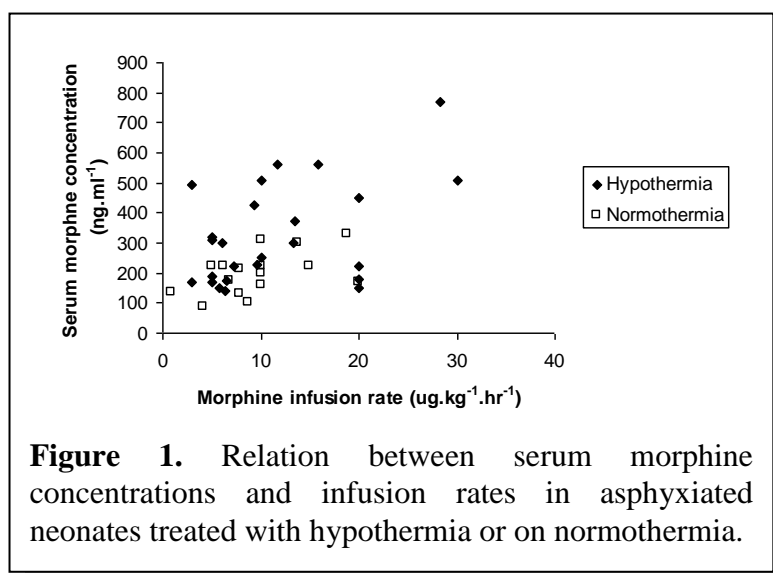
IL-6 levels (at 6 hours of age) (Figure 3.) and IL-4 levels (during first 24 hours) were significantly lower in asphyxiated neonates treated with hypothermia compared to normothermic neonates.

The duration of hypothermia correlated with lower levels of IL-6 (p=0.02), INF-γ(p=0.05), and TNF-α (p=0.04) levels measured at 6 hours of age and IL-10 levels at 12 hours (p=0.01). Cortisol levels did not differ significantly between neonates on hypothermia and normothermia at any time points. At 24 hours of age, 7/8 (87.5%) neonates in the normothermic, and 9/10 (90%) of the hypothermia group presented with cortisol levels lower than 414 nmol/L suggestive for relative adrenal insufficiency. At 72 hours of age neither significant differences between groups, nor significant correlation between cortisol and cytokine levels were observed.

Figure 3. Changes in serum IL-6 in hypothermic and normothermic infants. Median and interquartile range was shown. *, significantly different from normothermia (p<0.05), #, significantly different from values measured at 6 hours of age (p<0.05).

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Serum morphine concentrations at 24 to 72 hours after birth were (median [range]) 292 ng/mL (137–767 ng/mL) in the HT infants and 206 ng/mL (88–327 ng/mL) in the infants on normothermia, despite similar morphine infusion rates and cumulative doses. Morphine concentrations correlated with morphine infusion rate, cumulative dose, and treatment with hypothermia. Serum morphine concentrations reached a steady state after 24 hours in the NT infants but continued to increase throughout the assessment period in the HT group. Morphine clearance was low in both groups: (median [range]) morphine clearance estimated from area under the curve was 0.69 mL/min per kg (0.58–1.21 mL/min per kg) in hypothermic group and 0.89 mL/min per kg (0.65–1.33 mL/min per kg) in infants on normothermia. Serum morphine concentrations >300 nL/mL occurred more often in the hypothermia group and when the morphine infusion rate was >10 μg/kg per h.

Figure 1. Relation between serum morphine concentrations and infusion rates in asphyxiated neonates treated with hypothermia or on normothermia.

5. Conclusion

Our aim was to perform several observational studies on our study group while participating in the international, randomized TOBY trial. Our contribution helped to reach the desired patient number in the TOBY trial to answer the question about the efficacy of moderate systemic hypothermia treatment in HIE, and for us provided a reliable background for our publications. Since then, as hypothermia is clarified as standard care in HIE, it would be difficult to repeat our studies because of ethical reasons.

Our studies reported original data about comparing hypothermic and normothermic groups of term asphyxiated neonates in some aspects like markers of multiorgan failure and neuronal injury, inflammatory markers, and morphine metabolism.

1. We concluded first that systemic moderate hypothermia decreases the acute cell necrosis caused by hypoxic ischemic insult and may attenuate organ dysfunction in neonatal asphyxia.

2. We found that infants treated with moderate hypothermia had lower serum S100B levels compared with normothermic infants and serum NSE did not show clinically significant differences compared with the cooled infants. Serum S100B and NSE levels were significantly higher in the infants who died or had a severely abnormal neurological outcome but the association was stronger for serum S100B levels. These data suggest that serum S100B levels may be a useful biomarker of disease and treatment effect in studies of neuroprotective therapies following perinatal asphyxia.

3. Our other observation was that serum IL-6 levels were significantly lower in the hypothermia group at 6 hours of age suggesting that hypothermia may decrease immediately the early rise of IL-6 following asphyxia. Moreover a significant negative correlation between IL-6 levels at 6 hours of age and the duration of hypothermia, suggesting a "dose dependent" reducing effect of hypothermia on early rise of IL -6 serum levels.

4. Finally we published first that asphyxiated neonates treated with moderate systemic hypothermia receiving commonly used rates of morphine infusion for 72 hours developed higher and potentially toxic concentrations of morphine compared with normothermic infants, despite receiving similar cumulative morphine doses. These observations resulted changes in the analgesic treatment of infants treated with hypothermia, being more cautious about morphine dosage. Also has led to more extensive investigations about pharmacokinetics of frequently used drugs during hypothermia in neonates.

The main limitation of our studies is the very small patient number. However we included all neonates enrolled to the TOBY study during this period with available blood samples. Further studies would be required to confirm some of our findings, like the early changes seen in IL-6 levels, but this now may be difficult for ethical reason since moderate hypothermia is an accepted neuroprotective intervention for neonatal hypoxic-ischemic encephalopathy.

Continuation of data collection is vital for finding answers for the outstanding questions about hypothermia treatment in the future, and national cooling registers should be set up for these purposes – also in Hungary. Long term neurodevelopmental follow-up for these children also remains essential.

At the moment, several potential neuroprotective agents are waiting for human clinical trials in the next few years. As outcome measurements have improved, in the near future smaller clinical studies need to be carried out to assess the effectiveness of additional therapies combined with hypothermia. Brain MRI can be used as marker for brain injury, which can reduce the length of clinical studies assessing new therapies. New techniques like spectroscopy, diffusion weighted and diffusion tension imaging can assess the injury accurately and provide information about prognosis. However new MRI techniques for these newborns are often not available because of scanner availability and difficulties with transport. Serum biomarkers like S100B protein as we showed can also have future role in monitoring therapy effectiveness.

6. List of publications

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7. Acknowledgement

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Barna Vásárhelyi, my supervisor during the first PhD year was my first teacher in writing papers and he showed a lot of things not from the clinical but the research side. Although initially he had some doubt about the blond PhD student and my topic, this just encouraged me to work harder. He helped me through all my papers with great support.

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I am grateful to the infants and their parents whom I met during this period for their consent to participate in the research. I am very proud that I am still receiving e-mails and pictures of the boys and girls who were enrolled in the study or have received cooling since then, as they have grown older. It has been an amazing experience for me that after participating in the introduction of cooling therapy in Hungary, I was able to see these children recover and develop normally after the serious problems they experienced initially.