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ORIGINAL ARTICLE

# Does phenobarbital improve the effectiveness of therapeutic hypothermia in infants with hypoxic-ischemic encephalopathy?

**S Sarkar[1], JD Barks[1], JR Bapuraj[2], I Bhagat[3], RE Dechert[1], RE Schumacher[1] and SM Donn[1]**

*[1]Division of Neonatal-Perinatal Medicine, Department of Pediatrics, University of Michigan Health System, CS Mott Children's Hospital, Ann Arbor, MI, USA; [2]Division of Radiology, University of Michigan Health System, CS Mott Children's Hospital, Ann Arbor, MI, USA and [3]Department of Pediatrics, St Joseph Mercy Hospital, Ann Arbor, MI, USA*

**Objective:** To determine whether phenobarbital (PB) given before therapeutic hypothermia to infants with hypoxic-ischemic encephalopathy (HIE) augments the neuroprotective efficacy of hypothermia.

**Study Design:** Records of 68 asphyxiated infants of $\geqslant 36$ weeks' gestation, who received hypothermia for moderate or severe HIE were reviewed. Some of these infants received PB prophylactically or for clinical seizures. All surviving infants had later brain magnetic resonance imaging (MRI). The composite primary outcome of neonatal death related to HIE with worsening multiorgan dysfunction despite maximal treatment, and the presence of post-hypothermia brain MRI abnormalities consistent with hypoxic-ischemic brain injury, were compared between the infants who received PB before initiation of hypothermia (PB group, $n = 36$) and the infants who did not receive PB before or during hypothermia (No PB group, $n = 32$). Forward logistic regression analysis determined which of the pre-hypothermia clinical and laboratory variables predict the primary outcome.

**Result:** The two groups were similar for severity of asphyxia as assessed by Apgar scores, initial blood pH and base deficit, early neurologic examination, and presence of an intrapartum sentinel event. The composite primary outcome was more frequent in infants from the PB group (PB 78% versus No PB 44%, $P = 0.006$, odds ratio 4.5, 95% confidence interval 1.6 to 12.8). Multivariate analysis identified only the PB receipt before initiation of hypothermia ($P = 0.002$, odds ratio 9.5, 95% confidence interval 2.3 to 39.5), and placental abruption to be independently associated with a worse primary outcome.

**Conclusion:** PB treatment before cooling did not improve the composite outcome of neonatal death or the presence of an abnormal post-hypothermia brain MRI, but the long-term outcomes have not yet been evaluated.

Correspondence: Dr S Sarkar, Division of Neonatal-Perinatal Medicine, Department of Pediatrics, University of Michigan Health System, F5790 C.S. Mott Children's Hospital, 1500 East Medical Center Drive, Room F5790, Ann Arbor, MI 48109-0254, USA.
E-mail: subratas@med.umich.edu
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## Introduction

Currently, therapeutic hypothermia is the only intervention showing promise in improving neurologic recovery in term or late preterm newborn infants after hypoxic-ischemic encephalopathy (HIE), but it is not a 'silver bullet'.[1] Approximately one in nine infants (95% confidence interval 5 to 25) will garner some benefit from hypothermia.[2] One potential way to improve the effectiveness of neuroprotective treatment might be combining hypothermia with another neuroprotective agent.

Phenobarbital (PB) therapy, like hypothermia, provides nonspecific neuroprotection by targeting multiple sites in the cascade leading to hypoxic-ischemic brain injury. Barbiturates are believed to decrease cerebral metabolism and oxygen consumption, reduce post-ischemia calcium entry, and mitigate free radical damage during and immediately after hypoxia-ischemia.[3,4] However, it is not known if combining therapeutic hypothermia and PB improves neuroprotection for newborns with HIE.

We hypothesized that PB given to newborns with HIE before initiation of therapeutic hypothermia might have a synergistic neuroprotective effect.

The purpose of this retrospective study was to determine whether PB given before hypothermia improves short-term neuroprotection, defined as reduction in neonatal death related to HIE with multiorgan dysfunction, or reduction in the incidence of abnormal post-hypothermia brain magnetic resonance imaging (MRI) with imaging abnormalities consistent with hypoxic-ischemic injury.

## Methods

The University of Michigan investigators participated in the Cool Cap trial,[5] and now offer both selective head cooling (SHC)[5]

and whole body cooling (WBC),[6] with WBC now used more often.

In all, 86 consecutive cases of asphyxiated newborns of $\geqslant 36$ weeks' gestation, who received therapeutic hypothermia for moderate (Sarnat stage 2) or severe (Sarnat stage 3) HIE between December 2003 and February 2009 by either SHC, WBC or both were retrospectively reviewed. Clinical monitoring and treatment of the infants during therapeutic hypothermia, whether by SHC or WBC, were performed according to the Cool Cap and National Institute of Child Health and Human Development Neonatal Research Network cooling protocols.[5,6] Some of these infants also received PB prophylactically or for clinical seizures. Referring hospitals were discouraged from using prophylactic PB for clinical HIE without seizures. Neuromuscular agents were never used and use of sedation was rare during hypothermia treatment.

All infants receiving therapeutic hypothermia also underwent neuroimaging studies, usually MRI of the brain, at around 7 to 10 days of life per unit protocol. MRI was performed using a 1.5 T magnet with T1- and T2-weighted imaging sequences in both the transverse and sagittal planes. MRI scans were reviewed by neuroradiologists and pediatric neurologists during the initial admission to assess for the presence of abnormal signal intensities consistent with hypoxic-ischemic injury within the basal ganglia and thalamus, internal capsule, subcortical white matter or cortex. A brain MRI was abnormal if any of these were seen. Scans were later independently reviewed and categorized (for this study) by a neuroradiologist (JRB) masked to the clinical history, original MRI report, or outcome. The categorization of the brain injury was similar to basal ganglia/watershed scoring system proposed by Barkovich *et al*.,[7] reported to be predictive of neuromotor and cognitive outcome at 12 months, and were as follows: normal or no injury, abnormal signal in basal ganglia or thalamus, abnormal signal in cortex not extending beyond the watershed, abnormal signal in cortex not extending beyond the watershed and basal nuclei, abnormal signal in cortex extending beyond the watershed areas and basal nuclei.This categorization was based on reports of two basic categories of imaging patterns in asphyxiated infants, one with primary injury to the deep gray matter nuclei, and another in which the injury is primarily to the vascular boundary zones.[8]

The composite primary outcome of neonatal death related to HIE with worsening multiorgan dysfunction despite maximal treatment, or the incidence of abnormal post-hypothermia brain MRI with imaging abnormalities consistent with hypoxic-ischemic injury was compared between the infants who received PB before the initiation of therapeutic hypothermia and the infants who did not receive PB before or during therapeutic hypothermia. This composite outcome was selected because infants who died before undergoing brain MRI could not be otherwise classified.

*Statistical analysis*

Data were analyzed with commercially available statistical software (PASW 18, SPSS, Chicago, IL, USA). Clinical characteristics between groups were compared by $\chi^2$ tests for categorical data and *t*-test or Mann–Whitney test, as appropriate for continuous data. Two-sided *P*-values $<0.05$ were regarded as significant. The relations among all of the pre-hypothermia (before 6 h of age) clinical and laboratory variables, and the primary composite outcome were assessed by univariate analysis. The selected pre-hypothermia co-variates for this exploratory analysis included PB administration before therapeutic hypothermia; onset of clinical seizure before the start of therapeutic hypothermia; 5- and 10-min Apgar scores; pH of $<6.7$; base deficit of $>18.5\,\mathrm{mmol\,l^{-1}}$ or base deficit of $>22\,\mathrm{mmol\,l^{-1}}$ in cord blood, or during the first hour of life; presence or absence of specific components of an early neurologic examination at the initiation of hypothermia, including flaccidity, decerebrate posturing, absent spontaneous activity, and absent or weak suck/gag reflexes; mode of delivery; male gender; presence or absence of an intrapartum sentinel event (placental abruption, umbilical cord accident or/and uterine rupture, and so on); and history of meconium stained amniotic fluid with fetal bradycardia. The selection of these pre-hypothermia variables including pH and base deficit, and the specific components of the neurological examination to assess the severity of asphyxia was based on previous reports, and they were similar to those previously identified to be predictive of severe disability or death in infants with HIE despite therapeutic hypothermia.[9] Stepwise (forward), multivariate logistic regression was performed to determine which of the pre-hypothermia variables that were significant on univariate analysis were independently associated with the primary outcome of interest (neonatal death or abnormal brain MRI). We used specific components of the early neurologic examination at the initiation of hypothermia to compare the severity of HIE between the two groups because parts of the early neurologic evaluation (flaccidity, decerebrate posturing and absent spontaneous activity) are more predictive than Sarnat staging for death/severe disability in infants with HIE despite therapeutic hypothermia.[9] Given that point estimates for neuroprotective effects of therapeutic hypothermia are essentially identical between the large SHC and WBC trials,[2] the data from our study population receiving either SHC or WBC were merged for analysis.

Written informed consent was obtained from a parent before the start of cooling for all infants, and this retrospective review was approved by the Institutional Review Board at the University of Michigan.

## Results

The composite primary outcome measure was evaluated in 68 of the 86 cooled infants. Ten infants who received PB for clinical seizures developing after the initiation or completion of cooling,

and six infants who had only a computed tomographic scan of the brain and not MRI were excluded. Two additional infants, one who underwent veno-arterial extracorporeal membrane oxygenation for severe persistent pulmonary hypertension during cooling (confounding the interpretation of the MRI scan) and one who was later diagnosed with congenital myotonic dystrophy, were also excluded.

In all, 36 of the 68 study infants who received PB (mean PB loading dose: $24.4 \pm 6.3\,mg\,kg^{-1}$) before the initiation of therapeutic hypothermia comprised the hypothermia plus PB group (PB); two infants received prophylactic PB at the referring hospital soon after birth, and the other 34 infants received PB because of clinical seizure activity before the initiation of hypothermia. During 72 h of hypothermia treatment, 13 of these 34 infants were given additional PB because of recurrent seizure activity. Thirty-two infants who did not receive PB before or during 72 h of therapeutic hypothermia constitute the hypothermia alone group (No PB). One infant from the No PB group received midazolam for seizure activity before initiation of hypothermia.

The neonatal and obstetrical characteristics of the study population of 68 cooled infants are shown in Table 1. The pre-hypothermia variables to assess the severity of asphyxia, including Apgar scores, blood pH and base deficit in cord blood or within 60 min of birth, presence or absence of specific components of the neurologic examination at the initiation of hypothermia, and presence or absence of an intrapartum sentinel event were similar in the two groups (Table 1). The number of infants from both the groups who received selective head cooling or WBC was similar (Table 1). However, clinical seizures before the start of hypothermia were more common in the PB group compared with the No PB group (Table 1).

Ten infants (PB: 6 and No PB: 4) died from HIE with worsening multiorgan dysfunction despite maximal treatment during the first 4 weeks of life. The remaining 58 infants survived beyond the neonatal period and had MRI of the brain done at a median postnatal age of 8 days (interquartile range, 7 to 9). Median postnatal age at brain MRI acquisition was similar between the two groups (PB group 8 days, interquartile range 7 to 8 versus No PB group 8 days, interquartile range 7 to 9, $P = 0.510$). The presence of abnormal MRI signal intensities consistent with hypoxic-ischemic injury was no different whether reported on initial admission or later for this study. As is evident in Table 2, incidence of abnormal post-hypothermia brain MRI imaging abnormalities consistent with hypoxic-ischemic injury were more common in the surviving infants who received PB compared with the infants who did not (73% versus 36%, $P = 0.007$, odds ratio 4.9, 95% confidence interval 1.6 to 15.2). More surviving infants from the No PB group had normal MRI scans. The composite primary outcome of neonatal death related to HIE, or the incidence of abnormal post-hypothermia brain MRI were also more frequent in infants from the PB group compared with infants from the No PB group (Table 2). Table 3 shows the patterns of the cerebral lesions consistent with hypoxic-ischemic injury detected on post-hypothermia brain MRI.

Univariate analysis to examine which of the selected pre-hypothermia clinical and laboratory variables predict the primary outcome identified PB before hypothermia; an Apgar

**Table 1** Comparison of neonatal and maternal characteristics, and the method of therapeutic hypothermia between the PB versus No PB groups

| *Characteristics* | *PB (n = 36)* | *No PB (n = 32)* | P-*value* |
|---|---|---|---|
| *Neonatal* | | | |
| Birth weight (g) | 3185 ± 980 | 3182 ± 1107 | 0.748 |
| Gestational age (weeks) | 38.7 ± 1.6 | 38.8 ± 1.7 | 0.680 |
| Transferred from birth hospital | 34/36 (94) | 27/32 (84) | 0.241 |
| *5-min Apgar score* | | | |
| 0–3 | 28/36 (78) | 19/32 (59) | 0.121 |
| 4–6 | 7/36 (19) | 8/32 (25) | 0.770 |
| *10-min Apgar score* | | | |
| 0–3 | 18/36 (50) | 9/32 (28) | 0.085 |
| 4–6 | 16/36 (44) | 16/32(50) | 0.808 |
| *Blood gas within 60 min of birth* | | | |
| pH < 7.0 | 25/36 (72) | 17/32 (53) | 0.214 |
| pH ⩽ 6.7 | 10/36 (28) | 4/32 (13) | 0.144 |
| Base deficit > 18.5 mmol $l^{-1}$ | 23/36 (64) | 14/32 (44) | 0.143 |
| Base deficit > 22 mmol $l^{-1}$ | 16/36 (44) | 10/32 (31) | 0.322 |
| Male | 20/36 (55) | 21/32 (65) | 0.461 |
| Hypotonia | 22/36 (61) | 22/32 (69) | 0.614 |
| Decerebrate posturing | 9/36 (25) | 8/32 (25) | 1.000 |
| Absent spontaneous movement | 18/36 (50) | 9/32 (28) | 0.085 |
| Absent or weak suck or gag | 33/36 (91) | 24/32 (75) | 0.098 |
| Clinical seizure before cooling | 34/36 (94) | 1/32 (3) | < 0.0001 |
| *Maternal* | | | |
| Emergency cesarean section | 21/36 (58) | 22/32 (69) | 0.454 |
| MSAF | 11/36 (31) | 10/32 (31) | 1.000 |
| Abruption | 7/36 (19) | 8/32 (25) | 0.770 |
| Cord accident or uterine rupture | 7/36 (19) | 6/32 (19) | 1.000 |
| Home birth | 3/36 (8) | 0/32 (0) | 0.241 |
| Pregnancy-induced hypertension | 4/36 (11) | 1/32 (3) | 0.360 |
| *Method of hypothermia*[a] | | | |
| SHC | 20/36 (55) | 14/32 (44) | 0.466 |
| WBC | 15/36 (42) | 18/32 (56) | 0.331 |

Abbreviations: MSAF, meconium stained amniotic fluid; PB, phenobarbital; SHC, selective head cooling; WBC, whole body cooling.
[a]One infant from the PB group was initially started on SHC, but was subsequently switched to WBC because of malfunction of the SHC device.
Data are mean ± s.d., number of patients (%).

**Table 2** Short-term outcome of asphyxiated infants following treatment with therapeutic hypothermia plus PB versus therapeutic hypothermia alone

| *Outcomes* | *PB (n = 36)* | *No PB (n = 32)* | *P-value (OR, 95% CI)* |
|---|---|---|---|
| Death during neonatal period | 6/36 (17) | 4/32 (12) | 0.739 (1.4, 0.35–5.49) |
| Abnormal brain MRI in neonates surviving beyond 4 weeks | 22/30 (73) | 10/28 (36) | 0.007 (4.9, 1.6–15.2) |
| Death or abnormal brain MRI | 28/36 (78) | 14/32 (44) | 0.006 (4.5, 1.57–12.8) |

Abbreviations: CI, confidence interval; MRI, magnetic resonance imaging; OR, odds ratio; PB, phenobarbital.
Data are number of patients (%).
Abnormal brain MRI indicates presence of abnormal signal intensities consistent with hypoxic-ischemic injury on the MRI brain scans.

**Table 3** MRI results in 58 surviving infants who received 72 h of therapeutic hypothermia for HIE

| *Patterns of hypoxic-ischemic brain injury detected on post-hypothermia brain MRI* | *None (normal brain MRI)* | *Isolated BGT lesions* | *Isolated cortical lesions with or without subcortical WM lesions* | *BGT and cortical lesions not extending beyond watershade areas* | *BGT, cortical and subcortical white matter lesions extending beyond watershade areas* |
|---|---|---|---|---|---|
| *Infants surviving beyond neonatal period (n = 58)* | | | | | |
| PB ($n = 30$) | 8 | 4 | 8 | 2 | 8 |
| No PB ($n = 28$) | 18[a] | 1 | 5 | 0 | 4 |
| *Infants who died during neonatal period (n = 10, MRI: 6, No MRI: 4)*[b] | | | | | |
| PB ($n = 4$) | 0 | 0 | 0 | 1 | 3 |
| No PB ($n = 2$) | 0 | 0 | 1 | 0 | 1 |

Abbreviations: BGT, basal ganglia and thalamus; HIE, hypoxic-ischemic encephalopathy; MRI, magnetic resonance imaging; PB, phenobarbital; WM, white matter.
[a]One infant diagnosed with a chromosomal abnormality, and brain malformation but no lesions consistent with hypoxic-ischemic brain injury on post-hypothermia brain MRI.
[b]Four infants without brain MRI died during the first week of life before MRI could be done.
Data are number of patients. Normal brain MRI indicates absence of abnormalities consistent with hypoxic-ischemic injury.

score of 0 to 3 at 10 min, and an Apgar score of 4 to 6 at 10 min; base deficit of $>18.5\,mmol\,l^{-1}$ and base deficit of $>22\,mmol\,l^{-1}$; absent spontaneous movement; onset of clinical seizures before hypothermia; and placental abruption as being predictive of the primary outcome (Table 4). These covariates were included in the logistic regression using a forward multivariate logistic regression model. Of the co-variates entered into the model, only PB before hypothermia, an Apgar score of 4 to 6 at 10 min and placental abruption were independently associated with the composite primary outcome in the final model (Table 5). As the neonatal death rates were similar between the two groups (Table 2), forward multivariate logistic regression analysis was repeated for prediction of an abnormal brain MRI alone in the surviving infants to find out if the differences in the composite outcome were heavily weighted by imaging findings, and again, PB before hypothermia, an Apgar score of 4 to 6 at 10 min and placental abruption remained independently associated with the outcome of abnormal brain MRI (Table 6). Clinical seizures before the onset of therapeutic hypothermia were not independently associated with adverse outcome on either of the two multivariate analyses.

As the majority of the infants received PB for clinical seizures, forward multivariate logistic regression analysis was also repeated with all of the pre-hypothermia clinical and laboratory variables except PB for prediction of the primary outcome in all 68 study infants, and again the onset of clinical seizures before hypothermia was noted not to be independently associated with the primary outcome (Table 7).

## Discussion

This study, although from a single center and limited by its retrospective design, is the first to evaluate the short-term neuroprotective efficacy of combining therapeutic cooling with PB in a large cohort of newborn infants with HIE. Our results indicate that combining therapeutic hypothermia with PB was not associated with significant clinical benefit. On the contrary, PB given to infants with HIE before hypothermia was associated with an increase in HIE-related neonatal death or the presence of an abnormal post-hypothermia MRI brain scan. It may be argued that the infants who received PB for clinical seizures might have been destined for worse outcomes because of more severe initial brain injury. Indeed, seizures are common in infants with HIE and they may exacerbate hypoxic-ischemic brain injury in this population.[10,11] However, forward logistic regression analysis identified PB treatment and not clinical seizure activity to be independently associated with an adverse outcome. Even when

**Table 4** Univariate analysis of all selected pre-cooling attributes for prediction of primary outcome of composite neonatal death related to HIE or an abnormal post-cooling MRI brain scan consistent with hypoxic-ischemic injury in 68 cooled infants

| *Variables* | *Odds ratio (95% CI)* | P-*value* |
|---|---|---|
| Phenobarbital before start of hypothermia | 4.5 (1.6–12.9) | 0.006 |
| 5-min Apgar score of 0–3 | 1.3 (0.5–3.8) | 0.602 |
| 5-min Apgar score of 4–6 | 0.45 (0.1–1.4) | 0.231 |
| 10-min Apgar score of 0–3 | 6.6 (1.9–22.7) | 0.002 |
| 10-min Apgar score of 4–6 | 0.2 (0.05–0.5) | 0.001 |
| First blood pH of <7.0 within 60 min | 2.2 (0.8–6.1) | 0.132 |
| First blood pH of <6.7 within 60 min | 2.7 (0.68–10.9) | 0.219 |
| First base deficit of >18.5 mmol $l^{-1}$ within 60 min | 2.8 (1.04–7.9) | 0.048 |
| First base deficit of >22 mmol $l^{-1}$ within 60 min | 4.2 (1.3–13.2) | 0.020 |
| Clinical seizures before start of hypothermia | 3.1 (1.1–8.5) | 0.045 |
| Flaccidity | 2.1 (0.7–5.9) | 0.193 |
| Decerebrate posturing | 0.8 (0.3–2.6) | 0.781 |
| Absent spontaneous activity | 6.6 (1.9–22.7) | 0.002 |
| Absent or weak suck/gag reflexes | 2.2 (0.6–8.2) | 0.312 |
| Male | 0.4 (0.1–1.2) | 0.127 |
| Abruption placenta | 5.4 (1.1–26.2) | 0.034 |
| Cord accident or/and rupture uterus | 0.7 (0.2–2.3) | 0.540 |
| MSAF with fetal bradycardia | 1.0 (0.3–2.9) | 1.000 |

Abbreviations: CI, confidence interval; HIE, hypoxic-ischemic encephalopathy; MRI, magnetic resonance imaging; MSAF, meconium stained amniotic fluid.

**Table 5** Multivariate logistic regression analysis for prediction of primary composite outcome of neonatal death or presence of an abnormal brain MRI in 68 cooled infants

| *Variables* | *Odds ratio (95% CI)* | P-*value* |
|---|---|---|
| Phenobarbital load before start of cooling | 9.5 (2.3–39.5) | 0.002 |
| 10-min Apgar score of 4–6 | 0.12 (0.03–0.48) | 0.003 |
| Abruption | 10.3 (1.4–76.7) | 0.023 |

Abbreviations: CI, confidence interval; MRI, magnetic resonance imaging.

logistic regression analysis was repeated after exclusion of PB as one of the co-variates, clinical seizures were not an independent predictor of adverse primary outcome indicating a true negative effect of PB when combined with therapeutic hypothermia. Moreover, the results of this study are supported by a certain measure of biological plausibility. Barbiturates have been used prophylactically (whether or not the newborn has seizures) following perinatal asphyxia to prevent development of seizures and to improve neurodevelopmental outcomes.[12–14] However, there is little evidence that treatment is beneficial. No difference in risks of death, severe neurodevelopmental disability, or the combined outcome of death or severe neurodevelopmental disability could be demonstrated in a Cochrane review of the studies comparing barbiturates with conventional therapy following

**Table 6** Multivariate forward logistic regression analysis of all of the pre-hypothermia variables for prediction of an abnormal brain MRI in infants surviving beyond the neonatal period ($N = 58$)

| *Variables* | *Odds ratio (95% CI)* | P-*value* |
|---|---|---|
| Phenobarbital load before start of cooling | 9.3 (2.1–40.2) | 0.003 |
| 10-min Apgar score of 4–6 | 0.15 (0.03–0.64) | 0.010 |
| Abruption | 7.8 (1.0–60.5) | 0.049 |

Abbreviations: CI, confidence interval; MRI, magnetic resonance imaging.

**Table 7** Multivariate forward logistic regression analysis of all of the pre-hypothermia variables except phenobarbital for prediction of primary composite outcome of neonatal death or an abnormal brain MRI in 68 cooled infants

| *Variables* | *Odds ratio (95% CI)* | P-*value* |
|---|---|---|
| Clinical seizure before start of cooling | 3.2 (0.97–10.9) | 0.055 |
| 10-min Apgar score of 4–6 | 0.18 (0.05–0.6) | 0.006 |
| Absent spontaneous activity | 4.5 (1.2–17.3) | 0.025 |

Abbreviations: CI, confidence interval; MRI, magnetic resonance imaging.

perinatal asphyxia.[15] In a recent small retrospective analysis also no reduction in neurodevelopmental impairment was seen in cooled infants who received prophylactic PB.[16] Both animal studies and some observational studies in older infants and children suggest that PB may not be safe. Developing brains may be particularly vulnerable, and animal studies demonstrate that 'exposure to PB may lead to apoptotic neurodegeneration in the developing brain when used at concentrations similar to those in infants with seizures'.[17,18] The potential adverse effects of PB on the developing brain include not only apoptosis but also 'interference with cell proliferation and migration, axonal arborization, synaptogenesis and synaptic plasticity',[19] and all of these may potentially reduce the effectiveness of hypothermia treatment.

The patterns of brain injury detected on post-hypothermia brain MRI in this study were categorized using previously published classifications.[7] This study was not designed to look at the effect of combining PB with hypothermia on the pattern of brain injury but rather on the incidence of abnormal brain MRI following such treatment. Conventional MRI can be a useful biomarker and potential surrogate endpoint for therapeutic hypothermia studies, and infants without brain injury on post-hypothermia MRI scans largely had normal outcomes. Indeed, based on the secondary analysis from the TOBY trial, investigators suggest that 'MRI in the neonatal period is qualified as a biomarker of the disease and treatment response and might be of use in neuroprotective studies'.[20] However, it is important to recognize that in the absence of long-term neurodevelopmental follow-up, it is not clear whether residual MRI abnormalities after therapeutic hypothermia with or without PB represent the final neuropathology or injury that may still be evolving.

In summary, the combination of therapeutic hypothermia and PB in this population of infants with HIE did not improve outcome. Rather, the combination was associated with an increase in the composite outcome of neonatal death related to HIE and the incidence of abnormal post-hypothermia brain MRI. However, because of the limitations of the retrospective study design, it is impossible to exclude the possibility that infants at higher risk of adverse outcome received PB before therapeutic hypothermia, especially when on closer inspection of the results of the repeat logistic regression analysis with all of the co-variates except PB showing clinical seizures reaching close to 0.05 level of significance for prediction of the primary outcome, suggesting that the two infants who received prophylactic PB but did not seize, and one infant who received midazolam for seizure activity probably exerted significant leverage within the logistic regression model. Nevertheless, this study offers insights into the potential consequences of PB treatment during therapeutic hypothermia, emphasizes the caution needed with this approach and the need for a clinical randomized controlled trial and further large animal-model pre-clinical studies to assess the effectiveness of combining therapeutic hypothermia and PB for treatment of HIE. This is important as there appears to be a temptation for clinicians to administer PB early, particularly in light of a recent report describing the combination of therapeutic hypothermia and PB beneficial in an animal model.[21]

## Conflict of interest

The authors declare no conflict of interest.

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