学位論文

Relationship between prolonged neural suppression and cerebral hemodynamic dysfunction during hypothermia in asphyxiated piglets

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Relationship between prolonged neural suppression and cerebral hemodynamic dysfunction during hypothermia in asphyxiated piglets

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Abstract

Objectives: Hypothermia (HT) improves the outcome of neonatal hypoxic-ischemic encephalopathy. Here, we investigated changes during HT in cortical electrical activity using amplitude-integrated electroencephalography (aEEG) and in cerebral blood volume (CBV) and cerebral hemoglobin oxygen saturation using near-infrared time-resolved spectroscopy (TRS) and compared the results with those obtained during normothermia (NT) after a hypoxic-ischemic (HI) insult in a piglet model of asphyxia. We previously reported that a greater increase in CBV can indicate greater pressure-passive cerebral perfusion due to more severe brain injury and correlates with prolonged neural suppression during NT. We hypothesized that when energy metabolism is suppressed during HT, the cerebral hemodynamics of brains with severe injury would be suppressed to a greater extent, resulting in a greater decrease in CBV during HT that would correlate with prolonged neural suppression after insult.

Methods: Twenty-six piglets were divided into four groups: control with NT (C-NT, $n = 3$), control with HT (C-HT, $n = 3$), HI insult with NT (HI-NT, $n = 10$), and HI insult with HT (HI-HT, $n = 10$). TRS and aEEG were performed in all groups until 24 h after the insult. Piglets in the HI-HT group were maintained in a hypothermic state for 24 h after the insult.

Results: There was a positive linear correlation between changes in CBV at 1, 3, 6, and 12 h after the insult and low-amplitude aEEG (<5 μV) duration after insult in the HI-NT group, but a negative linear correlation between these two parameters at 6 and 12 h after the insult in the HI-HT group. The aEEG background score and low-amplitude EEG duration after the insult did not differ between these two groups.

Abbreviations: aEEG, amplitude-integrated electroencephalography; CBF, cerebral blood flow; CBV, cerebral blood volume; $CMRO_2$, rate of cerebral metabolism of oxygen; HI, hypoxic-ischemic; HIE, hypoxic-ischemic encephalopathy; HR, heart rate; HT, hypothermia; LAEEG, low-amplitude electroencephalography; NIRS, near-infrared spectroscopy; NT, normothermia; PaO_2, arterial oxygen tension; ScO_2, cerebral hemoglobin oxygen saturation; TRS, time-resolved spectroscopy

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Discussion and conclusion: A longer low-amplitude EEG duration after insult was associated with a greater CBV decrease during HT in the HI-HT group, suggesting that brains with more severe neural suppression could be more prone to HT-induced suppression of cerebral metabolism and circulation.

Keywords: Animal model; Cerebral blood volume; Cerebral hemoglobin oxygen saturation; Electroencephalography; Hypothermic therapy; Hypoxia-ischemia; Hypoxic-ischemic encephalopathy; Near-infrared time-resolved spectroscopy; Piglet

1. Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) is a notable cause of neonatal death and developmental psychomotor disorders. The incidence of neonatal HIE is 1.6–3.8 per 1000 newborns [1–4]. In Japan, the estimated incidence of moderate-to-severe HIE in neonates born from a gestational age of 37 weeks onward is 0.35 per 1000 live births [5]. Mild hypothermia (HT) therapy is recommended for neonates with HIE by current neonatal cardiopulmonary resuscitation guidelines [6,7]. However, in studies where HT was initiated within 6 h of birth and continued for 72 h, the incidence of death or disability decreased from 58% to 47%, representing a risk reduction of just 11% [8–10]. Hence, about 45–50% of these neonates who were given HT still had major disability, died due to global multi-organ injury, or died after redirection of care from life support due to severe brain injury. Therefore, many questions remain regarding the selection of candidates for HT and its optimal degree and duration [11], and other treatment strategies are still urgently needed. In this regard, the following should be considered: (1) the methods used to classify neonatal HIE severity during the 6 h after birth and to evaluate treatment strategies tailored to condition severity, and (2) the identification of patients with risk factors for poor prognosis during HT requiring additional treatment, such as cerebral circulatory support.

The most promising and clinically feasible early indicator of hypoxia-ischemia after birth is amplitude-integrated electroencephalography (aEEG) due to its ease of use, its noninvasiveness, and its high prognostic value as early as 6 h after birth [12]. However, some studies have questioned the sensitivity of aEEG for detecting such neonates because some infants may have "slowly" evolving injuries and these infants may have a normal early aEEG. In addition, the timing of the injury in the actual clinical situation is not known at the time of assessment using aEEG, and the primary cerebral energy failure from hypoxic-ischemia might already be improving, or the damage from delayed secondary energy failure might have already started [13]. This might explain why some infants have adverse short-term outcomes despite normal aEEG. Furthermore, hypothermia may alter the prognostic value of aEEG performed within 72 h of birth [14].

In contrast, near-infrared spectroscopy (NIRS) permits continuous bedside monitoring of cerebral hemodynamics and oxygen metabolism. Previous studies using NIRS in term neonates with severe HIE (treated with or without HT) revealed that changes in cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral hemoglobin oxygen saturation (ScO_2) could predict an adverse outcome [15–18]. These studies reported that an increase in ScO_2 in the 24 h after birth was a poor prognostic feature in neonates with HIE, even those receiving HT therapy. We previously evaluated CBV and ScO_2 by near-infrared time-resolved spectroscopy (TRS) in infants with neonatal HIE within 72 h of birth and found that an increase in CBV was associated with poor prognosis [18]. In addition, in a piglet model of HIE, we found a positive correlation between the length of the neural suppression and the CBV increase in the 6 h following the hypoxic-ischemic (HI) insult and a correlation between the CBV increase at this time and the severity of brain tissue injury when assessed 5 days later [19–20].

Thus, simultaneous evaluation of aEEG recordings and CBV would enable more accurate prognostication and possibly identify patients with risk factors for poor prognosis during HT requiring additional treatment. However, it is unclear how this relationship is altered by HT. We previously noted that increased CBV during NT after insult can indicate the severity of brain injury and correlates with prolonged neural suppression after insult, because CBF becomes pressure passive due to impaired cerebral circulatory autoregulation [20,21]. However, we hypothesized that, during HT, when the energy failure is more severe, cerebral hemodynamics would be more passive and suppressed, possibly resulting in a greater decrease in CBV that correlates with prolonged neural suppression after insult. This phenomenon could indicate more severe brain injury and correlate with prolonged neural suppression after insult.

We developed a piglet model of asphyxia using aEEG and CBV measurements to control the severity of the hypoxic insult [22]. This model provides a good supply of animals that not only survive a hypoxic insult, but

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also sustain a consistent degree of histopathologic damage. Therefore, the objective of this study was to examine changes in aEEG and CBV and ScO_2 as measured by near-infrared TRS during HT and to compare these changes with those observed during NT in our piglet model of neonatal HIE.

2. Methods

2.1 Animal procedures

The study protocol was approved by the Animal Care and Use Committee at Kagawa University. Twenty-six newborn piglets within 24 h of birth and weighing 1.5–2.1 kg were obtained for the study and divided into four groups: HI-insulted piglets with NT (HI-NT group, $n = 10$), HI-insulted piglets with HT (HI-HT group, $n = 10$), control piglets with NT (C-NT group, $n = 3$), and control piglets with HT (C-HT, $n = 3$).

2.2 Anesthesia, ventilation, and monitoring of physiologic variables

The piglets were initially anesthetized with 1%–2% isoflurane in air using a facemask. Each piglet was then intubated and mechanically ventilated using an infant ventilator. The umbilical vein and artery were cannulated using a neonatal umbilical catheter for drip infusion and blood pressure monitoring/blood sampling, respectively. After cannulation, pancuronium bromide was used at an initial dose of 0.1 mg/kg, followed by infusion at 0.1 mg/kg/h to induce paralysis. Fentanyl citrate was then administered at an initial dose of 10 μg/kg followed by infusion at 5 μg/kg/h for anesthesia. A maintenance solution of electrolytes plus 2.7% glucose (KN3B; Otsuka Pharmaceutical Co., Tokyo, Japan) was infused continuously at a rate of 4 mL/kg/h via the umbilical vein. Arterial blood samples were taken throughout the experiment at critical time points and when clinically indicated. Each piglet was then placed under a radiant warmer to maintain a mean (standard deviation [SD]) rectal temperature of 39.0 (0.5) °C. The inspired gas was prepared by mixing O_2 and N_2 gases to obtain the oxygen concentrations required for the experiment. Ventilation was adjusted to maintain the arterial oxygen tension (PaO_2) and arterial carbon dioxide tension within their normal ranges.

2.3 Near-infrared TRS and analysis

We used a portable three-wavelength near-infrared TRS system (TRS-10; Hamamatsu Photonics K.K., Hamamatsu, Japan) and attached a probe to the head of each piglet. The light emitter and detector optodes were positioned in the parietal region at an interoptode distance of 30 mm. The TRS system at our institution uses a time-correlated single-photon counting technique for detection and has been described in detail elsewhere [23,24]. The oxyhemoglobin and deoxyhemoglobin concentrations were calculated from their absorption coefficients using equations that assume that background absorption is due only to 85% (by volume) water. ScO_2 and CBV were calculated as described previously [20].

2.4 aEEG measurements

The aEEG measuring device used was the Nicolet One (Cardinal Health, Inc., Dublin, OH). Using this device, the signal is displayed on a semi-logarithmic scale at low speed (6 cm/h). Measurements were recorded at 1-s intervals. The gold-plated electrode discs were placed at the P3 and P4 positions (corresponding to the left and right parietal areas on the head). Low-amplitude EEG (LAEEG) was defined as a maximum amplitude <5 μV; the aEEG pattern was evaluated using the aEEG scoring system developed by Peeters-Scholte et al. [25], which ranges from 4 (normal) to 0 (worst) and integrates the aEEG background pattern with seizure activity. Five distinct patterns can be discriminated in the aEEG backgrounds of human term neonates: flat trace, burst suppression, continuous low voltage, discontinuous normal voltage, and continuous normal voltage. Seizure activity was categorized as follows: no seizures present; an irregular, spiky aEEG (confirmed as multifocal epilepsy on a standard EEG recording); single seizures (<3 seizures/h, with a maximal duration of 10 min each); repetitive seizures (>3 seizures/h); and status epilepticus (saw-tooth pattern) [26].

2.5 HI insult

Hypoxia was induced at least 120 min after induction of anesthesia by decreasing the fractional concentration of inspired oxygen (FiO_2) to 0.04. The hypoxic insult was continued for 30 min. The FiO_2 was decreased (in 0.01 decrements) to a minimum of 0.02 or increased (in 0.01 increments) during the insult to maintain the LAEEG at <5 μV, heart rate (HR) at >130 beats/min, and mean arterial pressure (MAP) at >60% of baseline. When the criteria for LAEEG, HR, or MAP were satisfied during the first 20 min of the insult, the FiO_2 was returned to 0.04. For the final 10 min of the 30 min insult, hypotension was induced by decreasing the FiO_2 until the MAP decreased to below 60% of baseline. The criteria for resuscitation after the first 20 min of the insult were as follows: If the CBV value dropped below 33% during the insult, the insult was stopped and resuscitation was started (change in CBV during insult = [value of CBV at end of insult – value of CBV before insult]/[maximum value of CBV during insult – value

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of CBV before insult] × 100 [%]), even if the MAP was not maintained below 60% of baseline for 10 min [22].

In both the HI-NT and HI-HT groups, hypoxia was terminated by resuscitation with 100% oxygen. A base excess below −5.0 mEq/L was corrected as far as possible by infusion of sodium bicarbonate to maintain a pH of 7.3–7.5. After 10 min of 100% oxygen, the ventilation rate and FiO_2 were gradually reduced (SaO_2 95%–98%).

2.6 Post-insult treatment

The piglets in all groups received mechanical ventilation for 24 h after resuscitation. Piglets in the C-NT and HI-NT groups were maintained after resuscitation at 39 (0.5) °C under a radiant heater. In the C-HT and HI-HT groups, whole-body HT was achieved using a cooling blanket (Medicool; MAC8, Inc., Tokyo, Japan) after resuscitation. The piglets were cooled to 34.0 (0.5) °C for 24 h. Esophageal temperature was used as the measure of body temperature. The incubator temperature was maintained at 28–32 °C.

2.7 Data analysis

Statistical analyses were performed with GraphPad Prism 5 J (GraphPad Software, La Jolla, CA). The physiologic parameters, blood gas, aEEG background score, CBV, and ScO_2 (Tables 1 and 2) in each group were compared using one-way ANOVA with Tukey's post hoc analysis and the values obtained at the different time points were compared with the baseline values using non-parametric repeated measures ANOVA on ranks. Within-subject regression analysis (Spearman's correlation coefficient by rank test) was used to examine the relationships of the LAEEG duration with the change in CBV and ScO_2 (Fig. 3). All results are expressed as the mean (SD). The level of statistical significance was set at $p < 0.05$ for all tests.

3. Results

3.1 Physiologic parameters

The mean (SD) body weights were 1683 (189) g in the C-NT group ($n = 3$; 1 male and 2 females), 1806 (12) g in the C-HT group ($n = 3$; 2 males, 1 female), 1804 (108) g in the HI-NT group ($n = 10$; 5 males, 5 females), and 1761 (216) g in the HI-HT group ($n = 10$; 7 males, 3 females). During insult, the mean change in the CBV (mL/100 g brain) was 0.64 (0.3) in the HI-NT group and 0.55 (0.7) in the HI-HT group and the mean duration of insult (min) was 43.7 (6.1) in the HI-NT group and 43.2 (8.1) in the HI-HT group.

Table 1A summarizes the results of the physiologic parameters in all groups before, during, and after the HI insult. Between HI insult groups, MAP were lowest at the end of insult in both the HI-NT and HI-HT groups, with the HR after insult significantly lower in the HI-HT than in the HI-NT group (at 1, 3, and 6 h after birth, $p < 0.05$). MAP gradually increased after insult in the HI-NT group, whereas the MAP of the HI-HT group increased within 3 h after insult followed by a gradual decrement from 6 h after insult. In particular, it was significantly higher within 3 h after insult in the HI-HT group than in the HI-NT group; subsequently, the MAP of the HI-HT group was lower than that of the HI-NT group.

3.2 Blood biochemistry

Blood pH after insult was lower in the HI-HT group than in the HI-NT group (at 1 h, $p < 0.01$; 24 h after insult, $p < 0.05$) (Table 1B). PaO_2 tended to be higher in the HI-HT group than in the HI-NT group (1 h after insult, $p < 0.05$); the same pattern was seen in the control groups. During HT, blood glucose tended to be higher in the C-HT and HI-HT groups than in the C-NT and HI-NT groups. The base excess level after insult was lower in the HI-HT group than in the HI-NT group (at 1 h, $p < 0.001$; 3 h, $p < 0.01$; 6 h and 24 h after insult, $p < 0.05$) and the lactate level was higher in the HI-HT than in the HI-NT group (at 3 h after insult, $p < 0.05$).

3.3 Cerebral neural activity

The mean (SD) LAEEG duration after insult (min) was not significantly different between the groups (HI-NT, 25.0 [5.7]; HI-HT, 23.1 [11.0]). Furthermore, there was no significant difference in the aEEG background score between these groups (Table 2).

3.4 CBV and ScO_2

Within 1 h after insult, some piglets with NT and HT showed an increased CBV with a subsequent decrease; the others just showed a decrease in the CBV (Fig. 1A and B). Regarding the absolute value of the CBV, the HI-HT group showed a significantly lower CBV after insult than at baseline (at 3, 6, 12, and 24 h after insult; Table 2). Therefore, we calculated the change in the CBV from the end of insult to evaluate how much the CBV changed after the insult. We found that HI-HT group piglets had a more rapid decrease in CBV (change in CBV from 0 h), especially within 6 h after insult, than those of the HI-NT group at the same time point.

The ScO_2 in both the HI-NT and HI-HT groups was at its lowest value at the end of insult and then recovered. Although their values at the same time point were not significantly different from each other, the change in ScO_2 from the end of insult was significantly smaller in the HI-HT group than in the HI-NT group (at 3, 6, 12, and 24 h after insult, $p < 0.05$; Fig. 2).

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Table 1A
Physiological parameters and blood glucose and hemoglobin before, at the end of, and at 1, 3, 6, 12, and 24 h after hypoxic-ischemic insult in all groups (C-NT, C-HT, HI-NT, HI-HT).

Parameter	Group	Baseline	0	1	3	6	12	24
Heart rate (bpm)	C-NT	222 (14)	213 (20)	209 (28)	226 (5)	201 (20)	224 (5)	195 (13)
	C-HT	193 (14)	190 (31)	199 (18)	209 (14)	217 (5)	195 (27)	205 (32)
	HI-NT	201 (31)	157 (37)$^{\dagger\dagger\dagger\#}$	249 (20)†††§	257 (21)$^{\dagger\dagger\dagger\#\S\S\S}$	240 (21)$^{\dagger\dagger\#\#}$	231 (25)†	219 (42)
	HI-HT	225 (27)	162 (22)†††	196 (25) †***	211 (10)***	212 (10)**	209 (15)	189 (9)††
MAP (mmHg)	C-NT	89.9 (3.8)	78.5 (6.6)	81.6 (5.1)	74.1 (8.0)	79.1 (8.0)	85.6 (8.4)	60.9 (7.0)
	C-HT	85.7 (6.6)	79.3 (7.4)	82.3 (3.8)	83.7 (1.2)	87.7 (8.1)	85.0 (11.0)	68.3 (9.1)
	HI-NT	77.8 (9.7)	51.3 (13.1)$^{\dagger\dagger\dagger\#\#\S\S}$	62.7 (5.8)$^{\dagger\dagger\#\S}$	60.9 (6.5)$^{\dagger\dagger\dagger\#\S\S\S}$	69.3 (12.3)§	68.7 (10.3)	67.6 (16.4)
	HI-HT	80.4 (14.7)	45.9 (8.1)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	72.1 (9.6)*	73.6 (8.4)**	68.6 (8.0)§	66.8 (12.2)†	63.8 (8.5)†
Rectal temperature (°C)	C-NT	38.1 (0.2)	38.2 (0.1)	38.6 (1.0)	38.6 (0.7)	38.8 (1.1)	38.4 (0.8)	38.3 (0.6)
	C-HT	37.8 (0.9)	37.9 (0.8)	34.0 (0.4)$^{\dagger\dagger\dagger\#\#\#***}$	34.0 (0.4)$^{\dagger\dagger\dagger\#\#\#***}$	33.7 (0.4)$^{\dagger\dagger\dagger\ \#\#\#***}$	33.2 (0.2)†††	34.4 (1.5)†††
	HI-NT	37.7 (0.9)	37.5 (1.0)	38.1 (0.5)	38.0 (0.7)	38.1 (0.7)	37.8 (0.5)	38.1 (0.7)
	HI-HT	37.4 (1.3)	37.2 (0.5)	33.3 (1.1) $^{\dagger\dagger\dagger\#\#\#***}$	33.9 (0.6) $^{\dagger\dagger\dagger\#\#\#***}$	33.9 (0.4) $^{\dagger\dagger\dagger\#\#\#***}$	33.8 (0.4) $^{\dagger\dagger\dagger\#\#\#***}$	33.9 (0.5) $^{\dagger\dagger\dagger\#\#\#***}$
Blood glucose (mg/dL)	C-NT	140.3 (19.1)	134.3 (16.9)	137.0 (15.6)	147.7 (22.9)	181.3 (33.9)	159.0 (61.4)	85.3 (5.5)
	C-HT	166 (30)	173 (34)	217 (33)	245 (52)	260 (49)	282 (28) †	164 (64)
	HI-NT	151 (23)	225 (66)†	231 (55)†	198 (62)	171 (48)	191 (69)	154 (76)
	HI-HT	152 (35)	212 (1 0 1)	227 (85)†	239 (74)††	223 (78)†	266 (1 1 7)†††	257 (1 2 5)†††
Hemoglobin (g/dL)	C-NT	9.8 (1.0)	10.0 (0.9)	9.7 (1.0)	10.1 (0.6)	10.0 (1.0)	10.2 (0.6)	8.6 (0.2)
	C-HT	9.9 (2.1)	10.3 (2.0)	11.4 (2.3)	12.5 (3.1) $^{\dagger\dagger\dagger\#\#\#}$	12.6 (3.5) $^{\dagger\dagger\dagger\#\#\#}$	13.0 (3.2) $^{\dagger\dagger\dagger\#\#\#}$	10.6 (1.8)
	HI-NT	10.5 (2.4)	11.1 (2.9)	10.7 (2.0)	11.2 (2.1)†	11.4 (2.4)	11.1 (1.9)	10.1 (1.8)
	HI-HT	9.8 (1.5)	10.3 (1.6)	10.7 (1.8)	11.4 (2.0)†††	12.3 (2.2)†††	12.3 (1.9)†††	12.1 (1.5)$^{\dagger\dagger\dagger\#*}$

Values are shown as means (standard deviation). Abbreviations: bpm, beats per minute; C, control; HI, hypoxic-ischemic; HT, hypothermia; MAP, mean arterial blood pressure; NT, normothermia; pH_a, arterial pH; $PaCO_2$, arterial PCO_2; PaO_2, arterial PO_2.
$^{\dagger}p < 0.05$, $^{\dagger\dagger}p > 0.01$, $^{\dagger\dagger\dagger}p > 0.001$ vs baseline; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs C-NT group at the same point; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$, $^{\S\S\S}p < 0.001$ vs C-HT group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs HI-NT group.

Table 1B
Arterial blood gas data before, at the end of, and at 1, 3, 6, 12, and 24 h after hypoxic-ischemic insult in all groups (C-NT, C-HT, HI-NT, HI-HT).

Parameter	Group	Baseline	0	1	3	6	12	24
pH_u	C-NT	7.50 (0.0)	7.52 (0.0)	7.51 (0.0)	7.51 (0.0)	7.48 (0.0)	7.50 (0.0)	7.42 (0.1)
	C-HT	7.43 (0.05)	7.44 (0.05)	7.39 (0.06)	7.42 (0.05)	7.43 (0.07)	7.44 (0.07)	7.41 (0.04)
	HI-NT	7.43 (0.05)	6.86 (0.08)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	7.31 (0.04)$^{\dagger\dagger\dagger\#\#}$	7.48 (0.05)	7.47 (0.06)†	7.46 (0.05)	7.50 (0.06)†
	HI-HT	7.44 (0.1)	6.79 (0.09)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	7.19 (0.10)$^{\dagger\dagger\dagger\#\#\#\$**}$	7.42 (0.09)	7.44 (0.05)	7.46 (0.04)	7.42 (0.06)*
$PaCO_2$ (mmHg)	C-NT	39.8 (3.4)	37.7 (3.7)	40.0 (1.8)	38.8 (5.0)	39.8 (1.6)	37.1 (1.9)	41.1 (9.6)
	C-HT	41.4 (5.4)	43.8 (5.1)	47.9 (8.8)	43.1 (3.9)	41.3 (5.4)	36.5 (4.6)	39.4 (1.2)
	HI-NT	44.9 (4.3)	32.1 (12.6)††	42.3 (6.3)	43.1 (5.3)	45.0 (7.5)	44.1 (5.3)*	36.7 (4.7)†
	HI-HT	40.6 (10.2)	41.6 (15.7)	44.7 (8.5)	41.0 (7.4)	42.5 (8.6)	38.4 (5.1)	38.5 (7.3)
PaO_2 (mmHg)	C-NT	90.2 (15.2)	95.5 (11.2)	91.7 (10.8)	91.5 (10.09	96.9 (11.7)	87.8 (22.4)	83.1 (23.5)
	C-HT	111.5 (9.7)	109.8 (16.8)$^{\#\#}$	109.4 (22.7)	109.2 (13.4)	111.7 (13.5)	101.5 (4.7)	107.0 (6.7)
	HI-NT	87.9 (10.6)	17.5 (5.6)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	91.6 (26.7)	86.5 (19.0)	83.2 (14.0)	80.5 (13.8)	86.1 (20.2)
	HI-HT	91.1 (16.8)	21.2 (5.0)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	115.1 (20.9)†	88.7 (25.5)	86.6 (27.0)	88.3 (31.5)	89.0 (24.3)
Lactate (mg/dL)	C-NT	23.3 (6.0)	23.7 (8.5)	20.3 (8.5)	22.0 (9.5)	29.0 (11.2)	33.0 (13.5)	37.0 (25.5)
	C-HT	18.8 (6.3)	19.3 (7.9)	21.3 (3.7)	23.0 (7.9)	28.5 (11.6)	36.8 (14.7)	47.0 (21.7)
	HI-NT	17.5 (5.2)	225.6 (21.2)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	114.7 (28.1)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	34.3 (11.7)†§	29.6 (8.9)†	40.9 (6.8)†	43.3 (14.4)†
	HI-HT	22.9 (7.9)	210.5 (23.0)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	129.8 (22.2)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	70.6 (32.6)$^{\dagger\dagger\dagger\#**}$	40.7 (27.0)†	48.2 (18.4)†	49.0 (11.5)†
Base excess (mmol/mL)	C-NT	7.0 (1.1)	7.5 (0.7)	8.1 (1.4)	7.2 (1.3)	5.7 (2.1)	5.0 (2.6)	2.2 (3.2)
	C-HT	6.1 (1.5)	5.3 (2.3)	3.1 (3.7)	3.6 (3.8)	2.7 (2.8)	1.0 (4.0) †	0.4 (2.9) †
	HI-NT	5.4 (2.3)	−26.9 (2.9)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	−4.9 (2.6)$^{\dagger\#\#\#\S}$	7.8 (1.5)§	8.1 (1.7)§§	6.0 (2.7)§	5.5 (2.6)
	HI-HT	2.8 (2.9)	−27.9 (1.9)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	−10.8 (4.0)$^{\dagger\dagger\dagger\#\#\#\S\S\S**}$	1.6 (3.7) $^{\#***}$	4.3 (3.2)*	3.4 (4.0)	0.4 (4.4) *

Values are shown as means (standard deviation). Abbreviations: bpm, beats per minute; C, control; HI, hypoxic-ischemic; HT, hypothermia; MAP, mean arterial blood pressure; NT, normothermia; pH_a, arterial pH; $PaCO_2$, arterial PCO_2; PaO_2, arterial PO_2.
$^{\dagger}p < 0.05$, $^{\dagger\dagger}p > 0.01$, $^{\dagger\dagger\dagger}p > 0.001$ vs baseline; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs C-NT group at the same point; $^{\$}p < 0.05$, $^{\S\S}p < 0.01$, $^{\S\S\S}p < 0.001$ vs C-HT group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs HI-NT group.

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Table 2
Background score in aEEG and CBV and ScO_2 values and changes in CBV and ScO_2 before, at the end of insult, and at 1, 3, 6, 12, and 24 h after insult in all groups (C-NT, C-HT, HI-NT, HI-HT).

		Baseline	0	1	3	6	12	24
Background score of aEEG	C-NT	3	3	3	3	3	3	3
	C-HT	3	3	3	3	3	3	3
	HI-NT	3 (3-4)	0 (0–0)	2 (1–3)	2 (1–3)	2.5 (1–3)	2 (0–3)	2 (1–3)
	HI-HT	3 (3–3)	0 (0–0)	3 (1–4)	3 (1–3)	3 (1–3)	2 (1–3)	2 (1–3)
CBV (mL/100 g brain tissue)	C-NT	5.07 (0.66)	4.98 (1.0)†	5.15 (1.0)	4.80 (0.9)	4.61 (0.3)	4.51 (0.2)	4.83 (0.8)
	C-HT	5.21 (0.7)	4.90 (0.76)	5.03 (0.9)	4.40 (0.4)	4.10 (0.2)†	3.80 (0.5)††	4.18 (0.2)
	HI-NT	4.82 (0.77)	5.51 (0.80)†	5.35 (0.87)†	4.74 (0.84)	4.50 (0.91)	4.44 (0.84)†	4.11 (0.69)†
	HI-HT	5.44 (0.87)	5.69 (0.59)	5.47 (0.77)	4.75 (0.61)†	3.99 (0.47)†††	3.92 (0.74)†††	3.84 (0.59)†††
ScO_2 (%)	C-NT	67.0 (4.0)	67.7 (2.0)	66.6 (5.0)	65.4 (5.1)§	64.0 (0.6)	61.7 (3.4)	60.1 (6.9)
	C-HT	66.2 (5.9)	66.3 (7.2)	66.9 (7.0)	63.0 (4.4)	58.3 (5.9)	59.3 (7.0)	57.3 (7.6)†
	HI-NT	73.6 (5.0)	28.8 (6.2)$^{\dagger\dagger\dagger\#\#\#\S\S\S}$	76.1 (6.4)	74.0 (7.1)§	74.4 (6.9) $^{\#\S\S}$	73.6 (8.4) $^{\#\S\S\S}$	72.9 (5.2) $^{\#\#\S\S\S}$
	HI-HT	75.5 (4.2)	35.8 (6.0)$^{\dagger\dagger\dagger\#\#\#\S\S\S\bullet}$	79.3 (4.7)$^{\dagger\#\#\S}$	73.6 (5.0)§	72.2 (5.1)§	69.6 (5.5)†§	71.6 (5.3) $^{\#\S\S\S}$
Changes in CBV from 0 h after insult	HI-NT	NA	0	−0.16 (0.7)	−0.78 (0.8)	−1.0 (0.9)	−1.07 (0.7)	−1.40 (0.4)
	HI-HT	NA	0	−0.23 (0.5)	−0.94 (0.4)	−1.70 (0.7)	−1.77 (0.7)$^{\bullet}$	−1.85 (0.7)
Changes in ScO_2 from 0 h after insult	HI-NT	NA	0	47.3 (6.7)	45.1 (5.3)	45.6 (5.5)	44.7 (6.3)	44.1 (3.8)
	HI-HT	NA	0	43.5 (6.7)	37.7 (6.9)$^{\bullet}$	36.4 (4.8)$^{\bullet\bullet\bullet}$	33.7 (6.2)$^{\bullet\bullet\bullet}$	35.8 (4.9)$^{\bullet\bullet\bullet}$

Background score values are shown as the median (range) and the CBV and ScO_2 are shown as means (standard deviation). Abbreviations: aEEG, amplitude integrated electroencephalography; C, control; CBV, cerebral blood volume; HT, hypothermia; NT, normothermia; ScO_2, cerebral hemoglobin oxygen saturation. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p > 0.01$, $^{\dagger\dagger\dagger}p > 0.001$ vs baseline; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs C-NT group at the same point; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$, $^{\S\S\S}p < 0.001$ vs C-HT group; $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$ vs HI-NT group.

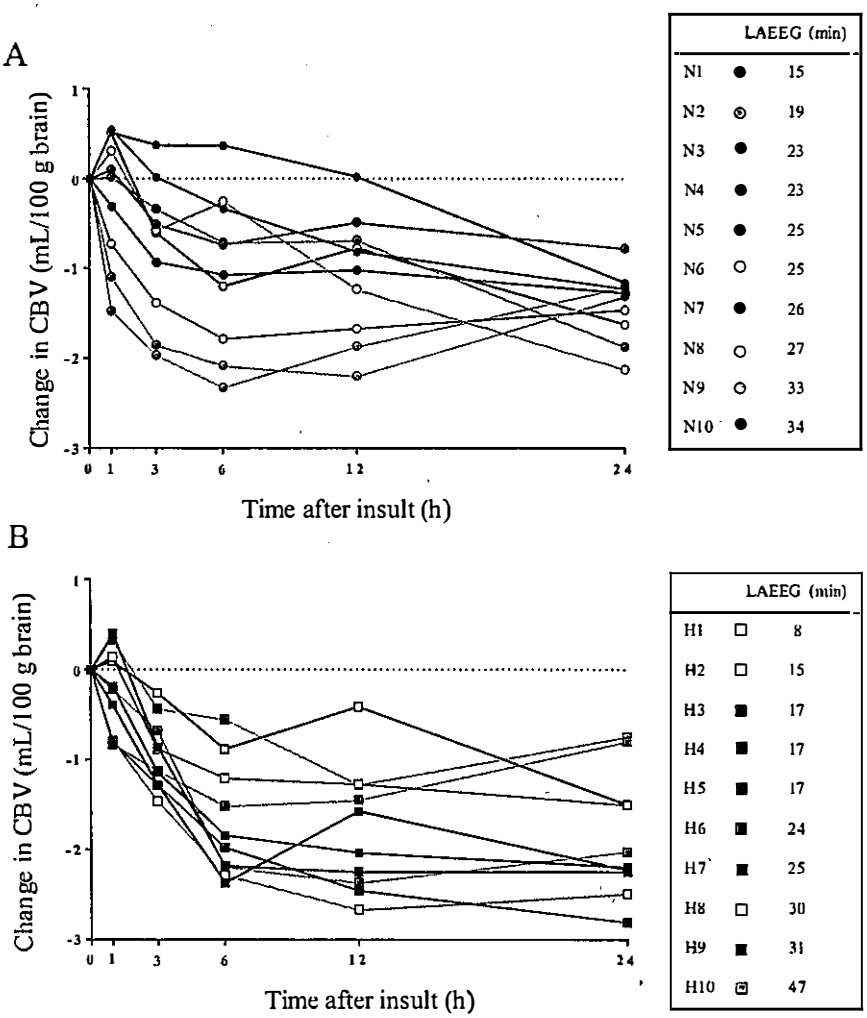

Fig. 1. Changes in CBV after the HI insult in the HI-NT group (A) and HI-HT group (B). The difference in CBV was calculated by subtracting the CBV value at the end of insult from the value at each time point.

3.5 Relationship between the CBV change and LAEEG duration after the insult

In the HI-HT group, some piglets with a longer LAEEG duration (H6-10; Fig. 1B) showed a tendency for a more rapid decrease in the CBV from 3 to 6 h after the insult compared with those with a shorter LAEEG duration (H1-4; Fig. 1B). Such a tendency was not seen in the HI-NT group. Furthermore, a significant positive correlation was observed between the change in the CBV and the duration of the LAEEG after insult (at 1 h, $p < 0.001$; at 3, 6, and 12 h, $p < 0.05$; Fig. 3). However, a negative correlation was observed after the insult in the HI-HT group (at 6 and 12 h, $p < 0.05$; Fig. 3). No correlation was observed between the LAEEG duration and the ScO_2 change after insult in either group.

4. Discussion

This study found (1) that CBV decreased within 24 h after insult in both groups of piglets and that this decrease was greater in the HI-HT group than in the HI-NT group; (2) a positive correlation between the CBV change and LAEEG duration after the insult in the HI-NT group, but a negative correlation between these parameters in the HI-HT group, and that MAP, HR, and blood gas data were similar between the groups at the end of insult; and (3) smaller differences in ScO_2 in the HI-HT group than in the HI-NT group after insult.

After the insult, the HI-HT group showed lower MAP and HR and higher blood glucose and hemoglobin during HT compared with the HI-NT group. The former showed lower pH and base excess and higher

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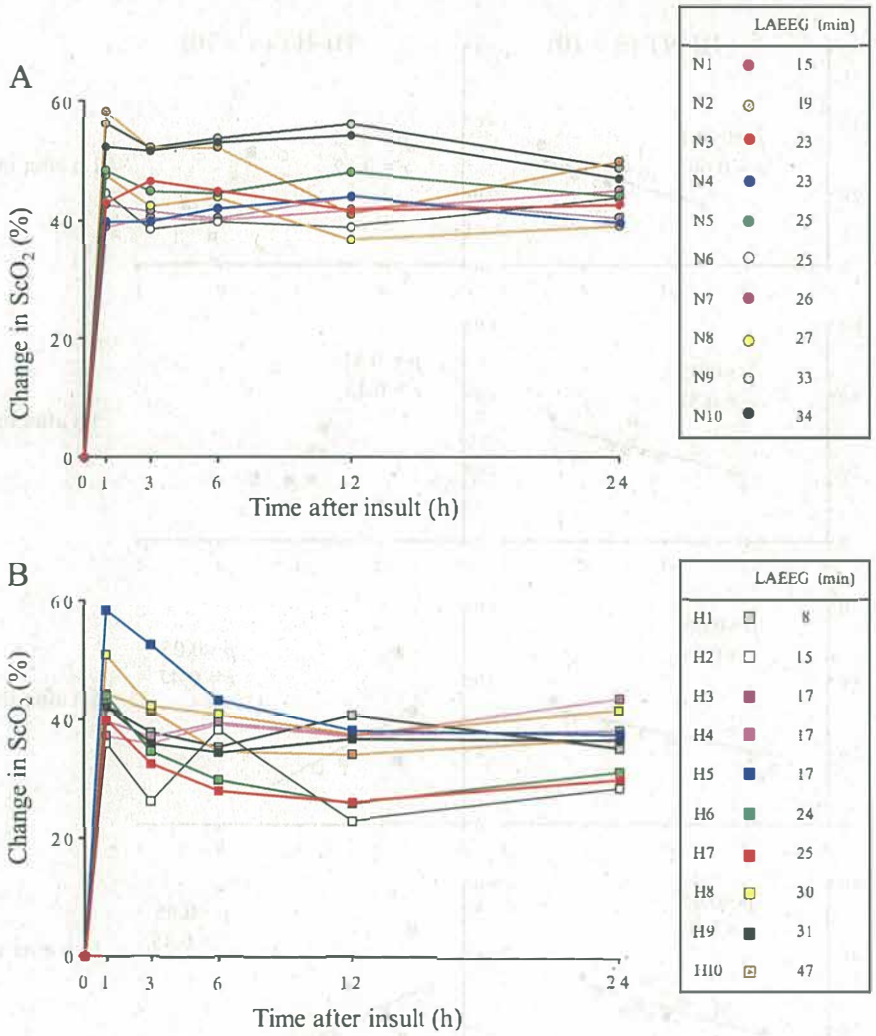

Fig. 2. Changes in ScO_2 after the HI insult in the HI-NT group (A) and HI-HT group (B). The difference in ScO_2 was calculated by subtracting the ScO_2 value at the end of insult from that at each time point.

lactate during HT compared with the other groups. We speculated that HT could lower cardiac output by suppressing cardiac function and could induce hyperglycemia, because HT can promote glycogenolysis and gluconeogenesis by stimulating adrenal catecholamines and glucocorticoids, and because insulin activity would be greatly reduced. Furthermore, HT increased cellular water production and decreased the plasma volume. It is generally believed that fluid is also sequestered in capillaries during HT; the addition of hemoconcentration and increased blood viscosity to an already compromised circulatory state results in severely impaired perfusion [27]. The higher lactate level during HT indicates that body metabolism is suppressed.

4.1 Effects of HT on CBV changes after insult

Compared with the HI-NT group, the HI-HT group had a greater decrease in CBV after insult. These results indicate that the CBV decreases more quickly during HT, possibly due to a greater decrease in CBF and the rate of cerebral metabolism of oxygen ($CMRO_2$). At 6–12 h after insult, more piglets had an increased CBV in the HI-NT group (6 of 10) than in the HI-HT group (2 of 10) (Fig. 1). These results may reflect the attenuation of cerebral hyperemia by HT. In a previous study, van Bel et al. [28] found that CBV decreased in neonates during HT performed as part of cardiopulmonary bypass surgery and suggested that this decrease might be due to decreased cerebral metabolic demands at a lower brain temperature. Reduction of the brain temperature to 35 °C decreases CBF and $CMRO_2$ when compared with a brain temperature of 39 °C [29], and every 1 °C decrement in brain temperature causes a 5% reduction in brain energy metabolism [30]. An important mechanism of HT-induced neuroprotection is the associated reduction in $CMRO_2$ [30]. Because cerebral circulation and cerebral metabolism are tightly

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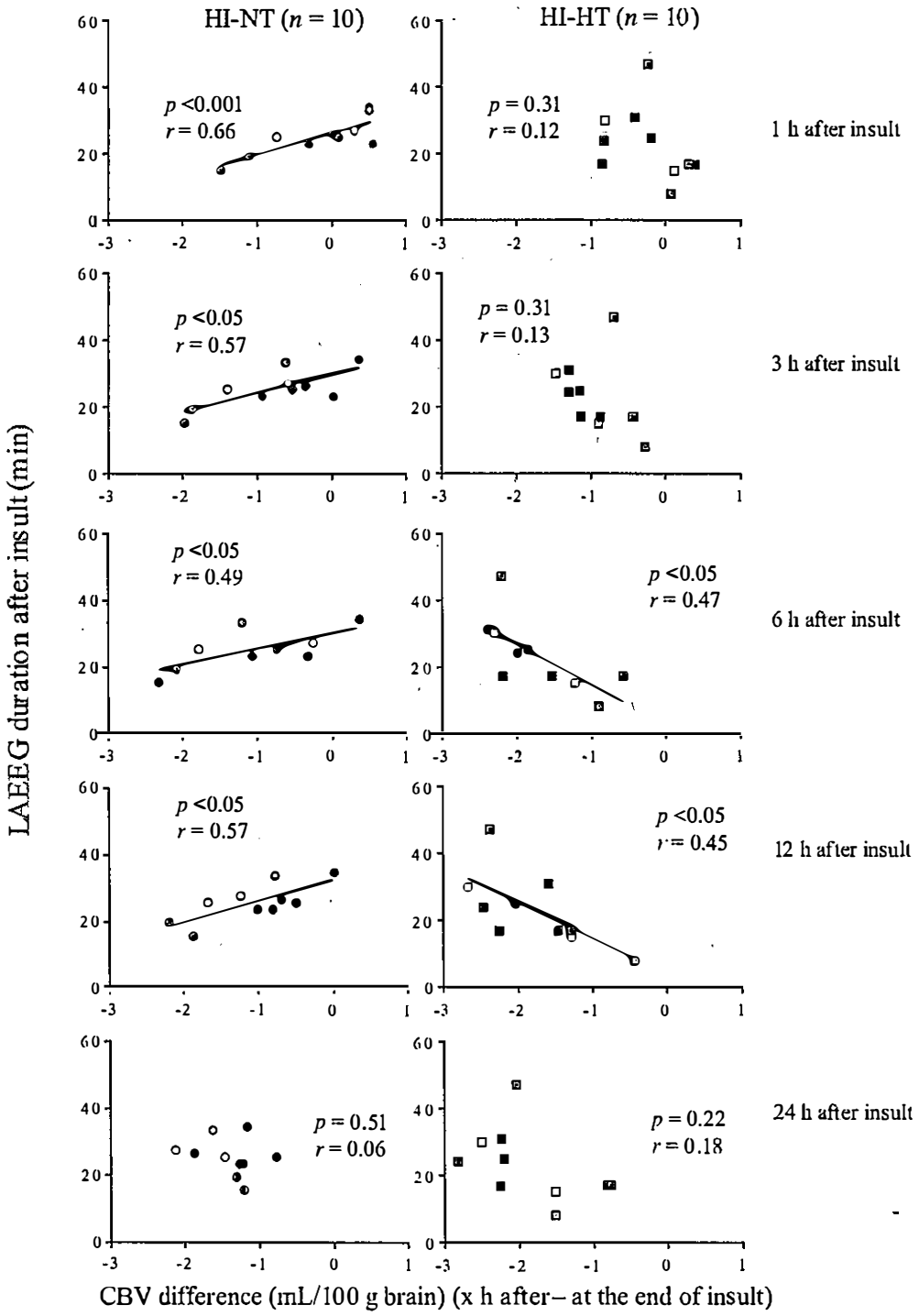

Fig. 3. Relationship between the changes in CBV and the duration of LAEEG from the end of insult to 1, 3, 6, 12, and 24 h after insult in the HI-NT and HI-HT groups. The difference in CBV and duration of LAEEG at each time point are plotted along the horizontal and vertical axes, respectively. Spearman's rank correlation coefficient was used, and the correlation coefficient (r) was calculated.

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regulated, a reduction in $CMRO_2$ typically results in decreased CBF [29].

4.2 Negative correlation between CBV changes and LAEEG duration during HT after insult

In this study, a negative correlation was found between the CBV change and the LAEEG duration after the insult in the HI-HT group, which is the opposite of the result in the HI-NT group. However, there was no significant difference in the post-insult aEEG background score or LAEEG duration between these two groups. Within 6 h after insult, which corresponds to the primary energy failure period, HI-HT group piglets with a longer LAEEG after the insult had a greater decrease in CBV. This result may indicate that brains with severe neural suppression could be more prone to HT-induced suppression of cerebral oxygen metabolism and oxygen consumption. Gunn et al. [31] reported on changes in cerebral electrical activity and CBF during HT in near-term fetal sheep. They found that fetuses exposed to HT sustained less brain damage, showed a CBF increase, and recovered from suppressive EEG activity within 24 h of the insult, suggesting that the increased CBF during HT may be related to less damage rather than a direct cooling effect. If this is the case, a greater CBV decrease and longer LAEEG duration during HT would indicate more severe cerebral neural and hemodynamic dysfunction.

Another possible explanation is that HT induces systemic circulation and the metabolism becomes more suppressed compared with NT, resulting in decreased CBF, which induces decreased CBV. With more severe brain injury, HT could accelerate the cerebral autoregulation impairment and make CBF more pressure passive. Therefore, for brains with prolonged neural suppression, which indicates severe brain injury, HT could reveal a different relationship between neural suppression and the change in CBV compared with NT.

Although many studies have reported that the CBF reduction induced by HT can be one of the normal physiological responses that reflects the reduction in the cerebral metabolic rate occurring with HT, we must discuss in detail whether this hypoperfusion exacerbates future brain injury or protects the brain due to the reduced metabolic demand [32].

4.3 Effects of HT on changes in the ScO_2 after an HI insult

A key finding of this study is that there was no significant difference in the duration of LAEEG during and after the HI insult between the HI-NT and HI-HT groups, even though there were differences in the ScO_2 and PaO_2 between the groups at the end of insult. However, this result might indicate lower oxygenation in the HI-NT group during insult because we performed the insult by controlling the percentage of oxygen using such parameters as duration of LAEEG and CBV and MAP, and not PaO_2 or ScO_2. Accordingly, we speculated that ScO_2 and PaO_2 during the HI insult might not always reflect neural suppression during and after the HI insult. Furthermore, we ensured that the two groups were exposed to the same degree of insult by using our protocol, irrespective of any difference in ScO_2 at the end of insult between these two groups.

Although the ScO_2 was restored to pre-insult levels after resuscitation, the difference in the ScO_2 was smaller in the HI-HT group than in the HI-NT group after the insult. ScO_2 is mainly influenced by cerebrovascular hemoglobin oxygen saturation, with contributions from the cerebral arterial, venous, and vascular beds. Presumably, the decrease in ScO_2 results from the greater hypoperfusion in the HI-HT group than in the HI-NT group, leading to a decreased arterial blood supply.

4.4. Limitations

We used fentanyl and pancuronium bromide as anesthesia. Previous animal studies using the newborn piglet model for HI insults were performed using a similar method of anesthesia. Although this anesthesia may reduce the cerebral circulation metabolic capacity, all groups were exposed to the same conditions, which is why their neural activity and background can be compared.

We investigated CBV and ScO_2, which can be measured simply and sequentially, but could not examine direct parameters related to the cerebral circulation or oxygen metabolism, such as CBF and $CMRO_2$. It is generally believed that HT therapy decreases CBF and $CMRO_2$, resulting in decreased CBV and increased ScO_2. To elucidate the pathophysiologic changes occurring during HT in patients with neonatal HIE, a simple method is needed for simultaneously measuring CBF and $CMRO_2$ at the bedside. Furthermore, we did not report histopathological results in this study. Therefore, we cannot speculate about the severity of brain injury in either of the NT and HT groups. We will examine other parameters together with histopathological results in future work.

5. Conclusion

In this piglet model of neonatal HIE, a longer duration of LAEEG after insult was associated with a smaller decrease in CBV in the HI-NT group but a greater decrease in CBV in the HI-HT group. We concluded that HT altered the relationship between the change in neural activity and cerebral hemodynamics and that brains with prolonged neural suppression had suppressed cerebral oxygen metabolism and hemodynamic

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control under HT. Therefore, the assessment of neural activity using aEEG together with monitoring of cerebral hemodynamics and oxygen metabolism using near-infrared TRS has the potential to determine the need for additional treatment for neonatal HIE when undertaking HT therapy.

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