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Cerebral blood volume measurement using near-infrared time-resolved spectroscopy and histopathological evaluation after hypoxic-ischemic insult in newborn piglets

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Thesis

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Cerebral blood volume measurement using near-infrared time-resolved spectroscopy and histopathological evaluation after hypoxic-ischemic insult in newborn piglets

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Abstract

The aim of this study was to assess the relationship between the cerebral blood volume (CBV) measured by near-infrared time-resolved spectroscopy (TRS) and pathological change of the brain in a hypoxic-ischemic (HI) piglet model.

Twenty-one anesthetized newborn piglets, including three sham controls, were studied. An HI event was induced by low inspired oxygen. CBV was measured using TRS (Hamamatsu TRS-10). Data were collected before, during, and 6 hours after the insult. CBV was calculated as the change from the end of the insult. The piglets were allowed to recover from anesthesia for 6 hours after the insult. At the age of 5 days, the brains of the piglets were perfusion-fixed, and histologic evaluations of brain tissue were performed. The extent of histopathological damage was graded in 0.5-unit intervals on a 9-step scale.

CBV increments were well correlated with histopathological scores, especially at 1 and 3 hours after resuscitation. Spearman's rank-correlation coefficients at 1, 3, and 6 hours after resuscitation in the gray matter were 0.9016, 0.9127, and 0.6907, respectively.

We conclude that an increased CBV after HI insult indicates more marked histological brain damage. CBV measurement immediately after resuscitation provides a more precise prediction of the histological outcome.

1. Introduction

In the therapeutic strategy for hypoxic-ischemic encephalopathy (HIE) associated with neonatal asphyxia, mild therapeutic hypothermia initiated early after birth, particularly within 6 hours, is effective, and evaluation in this period is very important [1–3]. Changes after hypoxic-ischemic (HI) insult in neonates and fetuses, including exacerbation of the amplitude-integrated electroencephalography (aEEG) pattern [2,3], an increase in the cerebral blood volume (CBV), an increase in the cerebral Hb oxygen saturation (ScO$_2$), and an increase in the cerebral blood flow (CBF), have been reported as factors indicating a poor prognosis clinically and by experimental studies using animals [4–9]. In particular, aEEG has been reported to be very useful for the prognosis, which is aggravated with a low amplitude, burst suppression, or flat trace on aEEG after hypoxic-ischemic insult [2,3,10]. However, as a low-amplitude pattern is often observed immediately after birth and gradually normalizes, the prognosis after about 6 hours is more accurate than that after 3 hours [11–13].

Regarding evaluation of the cerebral circulation using near-infrared spectroscopy (NIRS), Marks et al.[6], using a sheep fetus model of brain ischemia induced by carotid artery occlusion, showed that histological brain damage was severe when the changes in cerebral total Hb increased from about 12 hours after ischemic insult. Using NIRS and changes in the inspired oxygen concentration, Meek et al.[7] also reported that the CBV and CBF were increased during the first 24 hours in infants with adverse outcomes. While the timing of measurements after birth is not mentioned in their report, these findings are considered to reflect primarily delayed impairment of cerebral energy metabolism and subsequent cerebral circulation. In a clinical study in which the CBF was measured in term asphyxiated neonates using the xenon-133 clearance technique,

the prognosis was also unfavorable in children who showed increases in the CBF, which were considered to be due to vasoparalysis associated with brain damage [8]. On the other hand, van Vel et al.[14] observed in a clinical study that the CBV was reduced 2-12 hours after birth in neonates with marked asphyxia, so the results have not necessarily been consistent.

Many of the studies concerning evaluation of the cerebral circulation/oxygen metabolism after a hypoxic-ischemic event have been performed about 24 hours after the event or thereafter, and data concerning the cerebral circulation very early after insult are scarce. We have evaluated the relationships of aEEG findings with the CBV and ScO_2 early after hypoxic insult in an HIE model using newborn piglets[21].

We hypothesized that monitoring very early changes in the CBV from immediately after HI insult would help differentiate the severity of brain damage.

In this study, changes in the CBV and ScO_2 within 6 hours after resuscitation were compared with histological changes after 5 days.

2. Materials and Methods

2.1 Animal preparation

The study protocol was approved by the Animal Care and Use Committee of Kagawa University. Twenty-one newborn piglets, 12 males and 9 females, obtained within 24 hours of birth and weighing 1.63–2.10 kg, were used in this study. Eighteen piglets were subjected to HI insult and the other three were sham-controlled. The animal preparation method for the HI model was previously reported in detail [15].

Briefly, anesthesia was induced with 1–2% isoflurane in air using a facemask. Each piglet was then intubated and mechanically ventilated with a pressure-controlled, continuous flow infant ventilator (InfantStar Models 500, Infrasonics, San Diego, CA, USA). The umbilical vein and artery were cannulated with a neonatal umbilical catheter for drip infusion, and blood pressure monitoring and blood sampling, respectively. After cannulation, each piglet was paralyzed with pancuronium bromide at an initial dose of 0.1 mg/kg followed by infusion at 0.1 mg/kg/h, and then anesthetized with fentanyl citrate at an initial dose of 10 μg/kg followed by infusion at 5 μg/kg/h. Maintenance solution (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 at critical points and when clinically indicated throughout the experiment. Each piglet was then placed in a copper mesh-shielded cage under a radiant warmer to maintain a rectal temperature of 39.0 ± 0.5 degrees centigrade. 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 PaO_2 and $PaCO_2$ within their normal ranges. Arterial blood pressures were measured and recorded via the umbilical arterial catheter.

2.2 Near-infrared time-resolved spectroscopy and analysis

We used a portable three-wavelength near-infrared time-resolved spectroscopy (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 on the parietal region of each piglet with a 30-mm interoptode distance. In the TRS system, a time-correlated single-photon-counting technique is used for detection. The concentrations of oxyhemoglobin (oxyHb) and deoxyhemoglobin (deoxyHb) were calculated from the absorption coefficients of oxyHb and deoxyHb, with the assumption that background absorption was due only to 85% (by volume) water. The total cerebral Hb concentration [totalHb], ScO_2, and CBV were calculated as follows and also described previously [16,17]:

[totalHb] = [oxyHb] + [deoxyHb],

ScO_2(%) = {[oxyHb]/([oxyHb] +[deoxyHb])} ×100,

CBV (mL/100 g)= [totalHb] × MW_{Hb} × 10^{-6}/(tHb ×10^{-2}× D_t × 10),

where [ ] indicates the Hb concentration (μM), MW_{Hb} is the molecular weight of Hb (64,500), tHb is the blood Hb concentration (g/dL), and D_t is the brain tissue density (1.05 g/mL).

TRS measurements can be used to detect optical properties of brain tissue more selectively than continuous wave spectroscopy. Therefore, the influence of extracerebral tissue on this parameter in neonates might be comparatively small [18–20] .

2.3 Amplitude-integrated electroencephalography

Anesthetized piglets were placed in a copper mesh shield and then aEEG (Nicolet One; Cardinal Health, Inc., USA) was applied. All electrical devices and the copper mesh shield were grounded. The signal was displayed on a semi-logarithmic scale at a low speed (6 cm/h). We conducted measurements each second. Gold-plated electrode discs were placed at the P3 and P4 positions, which corresponded to the left and right parietal regions of the head. The maximum amplitude <5 μV was defined as low-amplitude EEG (LAEEG).

2.4 Hypoxic-ischemic insult protocol

Since the details were reported in our previous studies [15,21], only an outline of the HI insult protocol is presented here. Hypoxia was induced by reducing the inspired oxygen concentration of the ventilator to 4% after at least 120 min of stabilization from the initial anesthetic induction. If necessary to obtain a low-amplitude aEEG pattern (<5 μV), the inspired oxygen concentration was reduced further to 2%. From the beginning of LAEEG, the insult was continued for 30 min. FiO_2 was decreased (1% decrements) or increased (1% increments) during insult to maintain LAEEG, HR (>130 beats/min), and MABP (>70% of baseline). Upon the reinstatement of LAEEG, HR, or MABP, FiO_2 was returned to 4% during the first 20 min of insult. For the final 10 min of the 30 min insult, if MABP exceeded 70% of the baseline, hypotension was induced by decreasing FiO_2 until the MABP declined to below 70% of the baseline. Hypoxia was terminated by resuscitation with 100% oxygen. A base excess below -5.0 mEq/L was corrected as much as possible by sodium bicarbonate infusion to maintain a pH of 7.3–7.5. After 10 min of

100% FiO_2, the ventilator rate and FiO_2 were gradually reduced to maintain SaO_2 of 95–98%.

2.5 Post-insult treatment

  Once the piglets were weaned off the anesthesia and ventilator, and extubated 7–9 h after insult, they were allowed to recover and survived for 5 days in the incubator. Piglets were fed 50–80 mL of artificial animal milk via a nasogastric tube every 6 h. The temperature of the incubator was maintained at 28–32 degrees centigrade. The presence of seizures was recognized clinically as rhythmic pathologic movements (cycling) and tonic postures sustained between cycling episodes. If seizures occurred, the piglet was treated with phenobarbital (20 mg/kg) via intramuscular injection. If seizures persisted, the piglet was treated with two successive anticonvulsant doses. If seizures persisted after two successive anticonvulsant doses, the piglet was euthanized.

2.6 Histology

  After the 5-day period, the animals were initially anesthetized with isoflurane, and the brain of each animal was perfused with 0.9% saline and 4% phosphate-buffered paraformaldehyde. Histological evaluations of brain tissue were performed and irregularities were graded according to a histopathology grading scale for a piglet model of posthypoxic encephalopathy [22]. Coronal blocks of cortical gray matter, white matter, the hippocampus, and cerebellum were embedded in paraffin and cut with a microtome at 4 µm. The sections were stained with hematoxylin and eosin. At regular intervals of 100 µm, five sections each of samples were examined by M.U., who was blinded to all

clinical information. The extent of damage in each of the five regions was graded in 0.5-unit intervals on a 9-step scale that ranged from 0.0 to 4.0. We assigned a maximal score of 16 (cortical gray matter: 4.0; cortical white matter: 4.0; hippocampus: 4.0; cerebellum: 4.0) to experimental piglets that could not survive for 5 days due to severe seizures. This was because the minimum time for visible histologic damage to develop is shorter with increasing injury severity, and an increasing number of damaged neurons are visible from 6 to 24 hours [23,24]. Early evaluation might therefore cause underestimation of the damage.

2.7 Outcome grouping

Piglets were allocated to an outcome group based on the total histopathological score. Piglets with a total histopathological score of 0 were categorized as undamaged, while all histopathology scores of piglets that did not survive to 5 days were assigned as 4 (total score, 16) and they were categorized as dead. All other piglets were designated as damaged [15,22].

2.8 Data analysis

GraphPad Prism 5.01 for Windows software was used for all statistical analysis. As histpathology scores are not continuous but ordered variables, correlations among the CBV, ScO_2, and histopathology scores were compared using Spearman's rank correlation coefficient. We eliminated the dead piglets' results from the correlation calculation among CBV, ScO_2, and HIST scores. Wilcoxon's signed-rank test and the unpaired t-test were used for physiological parameters. All measurement results are

expressed as means ± standard deviation (S.D.). Values of < 0.05 were considered

significant.

3. Results

3.1 Physiological data

HR, MABP, and levels of arterial pH (pHa), PaO_2, $PaCO_2$, blood glucose, lactate, and hemoglobin in the piglets are shown in Table 1.

In the insult group, the HR, MABP, pHa, $PaCO_2$, PaO_2, base excess, and ScO_2 values were markedly decreased at the completion of HI insult, while blood glucose and lactate levels were markedly increased. These values generally returned to the baseline with time, whereas MABP, blood glucose, and lactate had not returned at 6 hours after resuscitation.

Table 1. Measurements of physiological, blood sampling, and NIRS variables before and at the end of insult, and 1,3,and 6 hours post-insult.

	Baseline		Peak CBV	End of insult		1 h		3 h		6 h	
	HI	Control	HI	HI	Control	HI	Control	HI	Control	HI	Control
Heart Rate, bpm	242±26#	207±12		131±26*#	213±29	254±30	290±12	261±27	265±49	227±34	232±28
MABP, mmHg	83±10	84±12		42±7*#	79±13	71±10*	79±8	68±9*	78±11	63±10*	69±2
pHa	7.46±0.08	7.46±0.07		6.93±0.10*#	7.38±0.04	7.32±0.09*	7.44±0.11	7.49±0.07#	7.39±0.04	7.49±0.08	7.47±0.07
$PaCO_2$, mmHg	40.6±7	39.0±7		29.5±6*#	48.9±6	35.5±8	43.3±0.3	37.0±3#	47.7±7	39.4±12	37.8±4
PaO_2, mmHg	109.3±24	127.0±32		15.4±4*#	82.0±11	100.3±28	89.2±5	89.2±23	81.6±13	99.0±24	104.0±7
Base excess, mmol/L	4.3±1.9	1.9±3.2		-25.6±3.9*#	2.8±0.8	-6.1±3.2*#	3.8±1.8	5.2±3.2	3.2±0.7	5.7±2.7	4.1±2.6
Blood glucose, mg/dL	153.4±40	125.7±44		223.7±102*	125.7±8	226.8±73*	142.3±22	187.7±62*	155.7±29	177.1±45*	154.3±48
Lactate, mg/dL	21.0±9	21.0±2		202.3±28*#	17.7±6	104.8±21*#	20.3±11	35.9±21*	17.7±4	34.4±16*	28.0±3
Hb, g/dL	10.1±1.7	8.9±2.3		10.5±1.8*	9.1±2.4	10.4±1.7	9.5±2.7	10.3±1.7	9.9±3.3	10.1±1.8	9.2±3.0
CBV, mL/100 g	5.5±0.8	5.5±0.1	7.4±0.9*	5.8±1.2	5.4±0.4	5.5±0.7	5.4±0.4	5.2±0.7*	5.5±0.5	5.1±0.9*	4.9±0.2
ScO_2,%	74.4±3.8	70.2±3.4	32.0±6.3*$	33.8±5.8*#	68.6±3.7	76.0±5.0#	68.5±4.2	73.7±4.4	68.5±2.0	74.2±3.6#	64.9±3.0

bpm, beats per minute; MABP, mean arterial blood pressure; pHa, arterial pH; $PaCO_2$, arterial PCO_2; PaO_2, arterial PO_2; CBV, cerebral blood volume; ScO_2, cerebral O_2 saturation.
*$p<0.05$ vs. preinsult basal level using Wilcoxon's signed-rank test. End of insult indicates the point at which resuscitation was started after HI insult. Values are means and SDs.
#$P<0.05$ vs. control (n=3) at the same point using unpaired t-test.
$$ScO_2$ value at peak CBV point.

3.2 Histological data and scoring

Table 2 shows the histological (HIST) scores of each piglet. Two piglets were undamaged, 11 were damaged, and 5 were dead. Examples of scored histologies are shown in Figure 1.
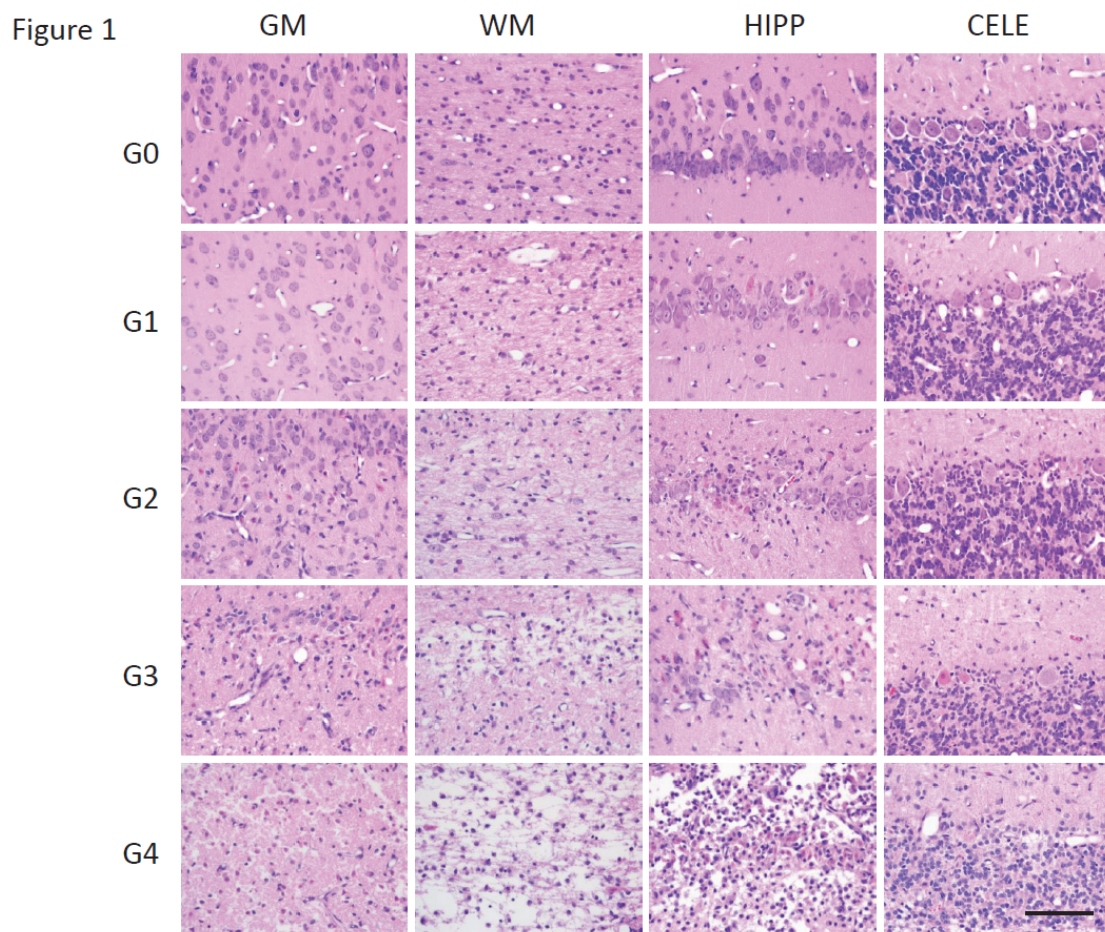
Figure 1.

Image of histological score for each part. Hematoxylin and eosin staining in the gray matter, white matter, hippocampus, and cerebellum. Scale bar = 100 μm. G0, G1, G2, G3, and G4 indicate graded histological scores developed by Thoresen et al. (1996).[22]

Table 2. Histopathology score for each piglet.

Piglet ID	Prognosis	GM	WM	HIPP	CERE	Total
a	undamaged	0	0	0	0	0
b	undamaged	0	0	0	0	0
c	damaged	1.4	1.8	1.6	0.6	5.4
f	damaged	2.6	2	1	0	5.6
e	damaged	2.2	1.9	1.2	0.4	5.7
g	damaged	3.6	3.4	3.8	0	10.8
l	damaged	3.8	3.8	3.2	0	10.8
j	damaged	3.6	3.6	3.4	0.6	11.2
d	damaged	3	3.3	3.8	1.8	11.9
k	damaged	3.6	3.6	4	1.8	13
h	damaged	3.7	2.8	4	2.6	13.1
m	damaged	4	4	4	2	14
i	damaged	3.8	3.8	4	2.8	14.4
n	dead	4	4	4	4	16
o	dead	4	4	4	4	16
p	dead	4	4	4	4	16
q	dead	4	4	4	4	16
r	dead	4	4	4	4	16

GM: gray matter, WM: white matter, HIPP: hippocampus, CERE: cerebellum

3.3 Cerebral blood volume and cerebral Hb oxygen saturation

In the HI group, the ScO_2 at the end of insult was significantly lower than in the control group. However, the CBV at the end of insult did not significantly differ between the two groups. In the HI group, the ScO_2 was low in all animals during hypoxia, but the CBV increased temporarily to a peak and decreased thereafter. The CBV was higher in the HI group during the insult, but showed no difference between the two groups at the end of the insult.

In the HI group, the CBV was reduced after 3 and 6 hours compared with the baseline. The ScO_2 was higher than in the control group after 1 and 6 hours. Even the control group presented a lower CBV at 6 hours compared with the baseline; however, no significance was shown (Table 1).

Figure 2 shows changes in the CBV, TRS, and aEEG in a typical case during the experiment. Those in histologically damaged, undamaged, and control piglet are shown in Figure 2a upper, Figure 2b middle, and Figure 2c lower, respectively.
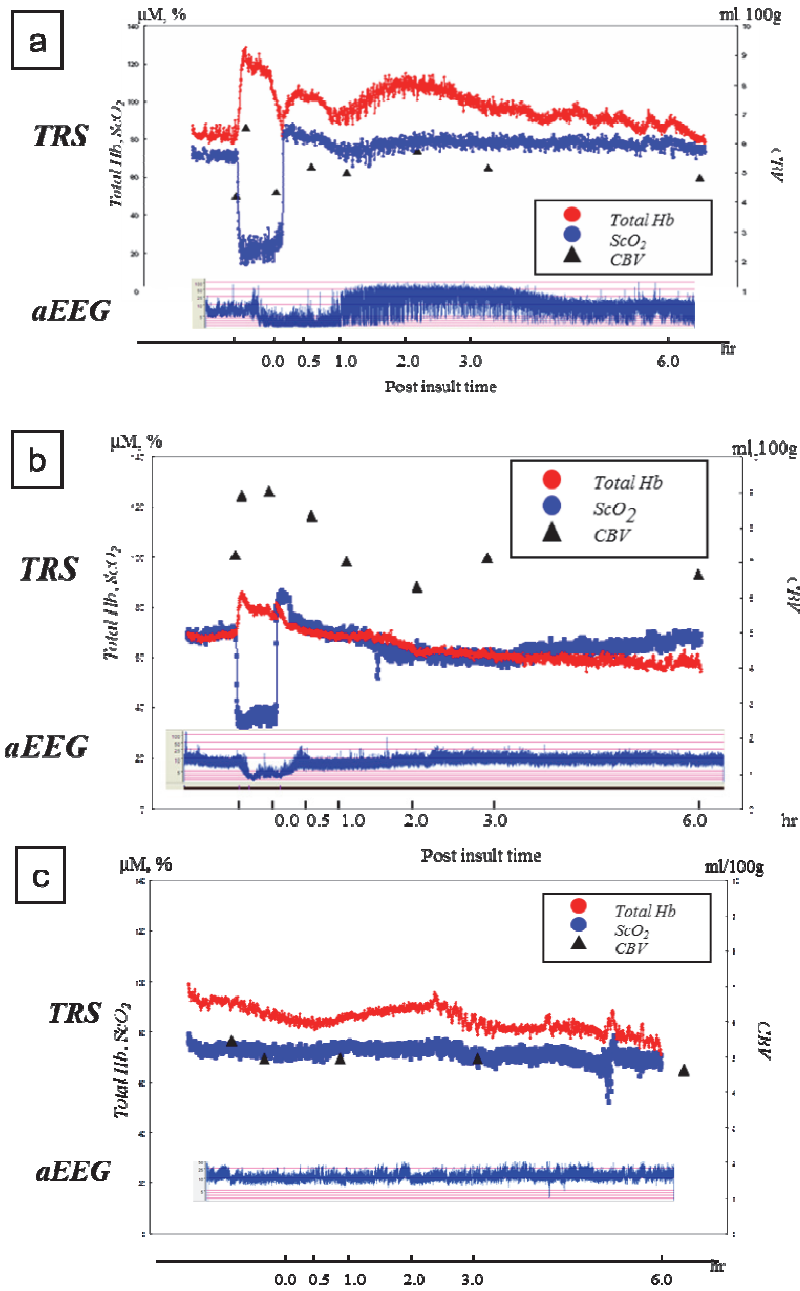
Figure 2

Figure 2. Typical patterns of TRS and aEEG throughout the experiment.

Figure 2a shows typical patterns of a piglet with an increased CBV and a poor prognosis.

The CBV increased after the beginning of HI insult and decreased gradually after a peak, but increased gradually again after the beginning of resuscitation (CBV increase pattern). aEEG after resuscitation also showed a burst suppression pattern and the outcome was poor.

Figure 2b shows typical patterns of a piglet with a decreased CBV and favorable prognosis.

The CBV increased due to HI insult, remained high until resuscitation was initiated and decreased gradually thereafter. After resuscitation, aEEG recovered from the low-amplitude pattern and the outcome was favorable.

Figure 2c shows typical patterns of a piglet of the sham control group.

The CBV decreased gradually towards the 6-hour point, however, no significance was shown at 6 hours compared with the baseline.

The CBV (5.5 ± 0.8 mL/100 g at baseline value) increased for a period after the beginning of HI, reached a peak (7.4 ± 0.9 mL/100 g), and then decreased (5.8 ± 1.2 mL/100 g) thereafter. In the undamaged newborn piglets in which the CBV was near the peak or above the baseline at the beginning of resuscitation, the CBV decreased after the resuscitation and the histological damage was mild (Figure 3a). On the other hand, in the damaged piglets, when resuscitation was initiated after the CBV increased to a peak and then decreased to a level near or below the baseline, the CBV increased again after resuscitation, and the histological damage was severe or the piglets died (Figure 3b). The transitions of the CBV and ScO_2 during HI insult were reported previously in detail [15,21].
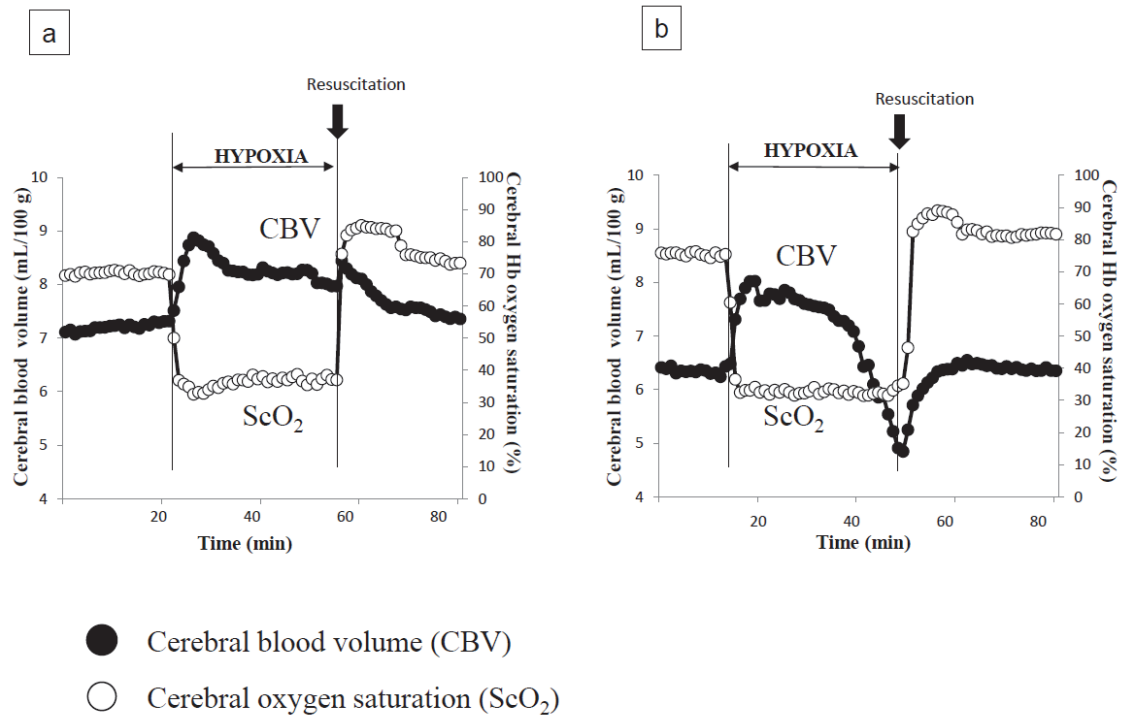
Figure 3

Figure 3. Typical transitions in CBV and ScO_2 before and during HI insult. The closed circle indicates CBV and open circle indicates ScO_2.

In Figure 3a, showing the example of a histologically undamaged piglet, CBV at the end of insult was higher than the baseline value and it decreased after resuscitation.   In Figure 3b, showing a damaged piglet, CBV at the end of insult was less than the baseline value, and it increased after resuscitation.

In the undamaged group, the CBV decreased rapidly in all animals after the initiation of resuscitation. In the dead group, however, the CBV increased from immediately after the beginning of resuscitation. In the damaged group, the CBV increased in some but decreased in others (Figure 4).

Figure 4. Changes in the CBV during the 6 hours after the end of insult.
The CBV decreased rapidly in the 2 histologically undamaged piglets and was reduced by 2 mL/100 g or more after 6 hours. However, the CBV increased in the piglets that died. It increased temporarily but soon began to decrease in those that died early.

The ScO_2 that was reduced by the HI insult increased from immediately after the beginning of resuscitation and returned to, or rose slightly above, the baseline, but no relationship was observed between the outcome and changes in the ScO_2 (Figure 5).
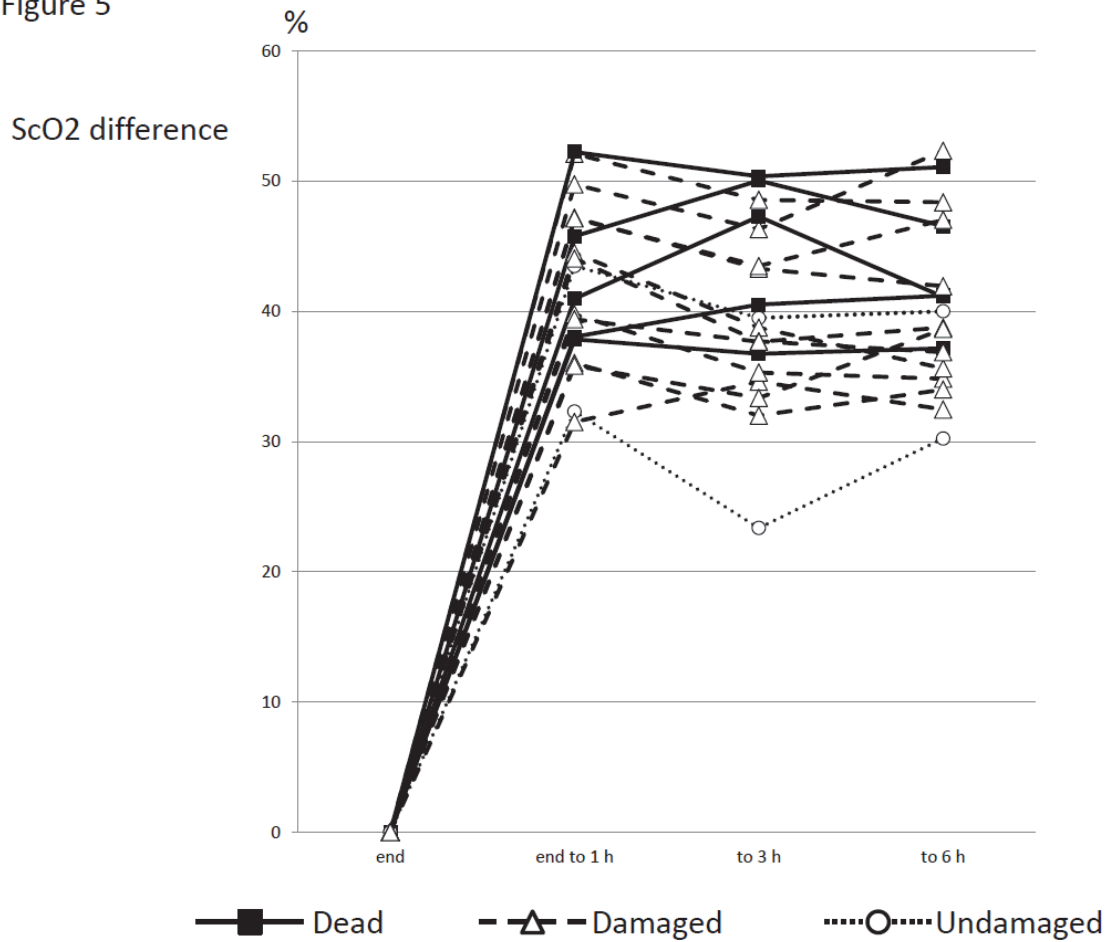
Figure 5

Figure 5. Changes in the ScO_2 over 6 hours after the end of insult.
The ScO_2 was increased 1, 3, and 6 hours after the end of insult in all animals. No relationship between the pattern of increase and outcome was observed.

3.4 Correlations among the CBV, ScO_2, and HIST scores

Figure 6 and Table 3 show the relationship of the change in the CBV with the HIST score at each site and time point. Increases and decreases in the CBV are shown with

positive and negative values, respectively. The figure shows the relationship between the changes in CBV and HIST scores at 1 hour after resuscitation. In the newborn piglets with positive CBV changes (increases) from immediately after resuscitation, high HIST scores and poor histological outcomes were shown. On the other hand, those with negative CBV changes (decreases) showed low HIST scores and mild tissue damage.
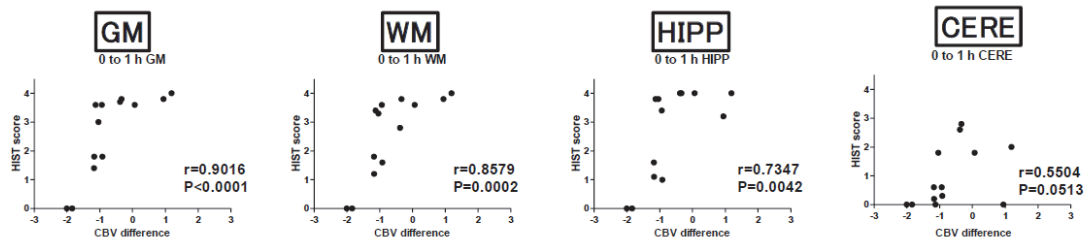
Figure 6

Figure 6. Relationship between the CBV change and histopathology score from the end of insult to 1 h after for white matter, gray matter, the hippocampus, and cerebellum.

The histopathology score and changes in the CBV in the gray matter, white matter, hippocampus, and cerebellum 1 hour after resuscitation are plotted along the vertical and horizontal axes, respectively. Spearman's rank correlation coefficient was used, and the correlation coefficient r was calculated. In all tissues, the correlation coefficient was high 1 hour after resuscitation.

Table 3. Correlation coefficients between CBV and HIST scores in different parts of the brain.

	GM	WM	HIPP	CERE	TOTAL
0 to 1 h	0.9016**	0.8579**	0.7347*	0.5504	0.7824*
0 to 3 h	0.9127**	0.8993**	0.8437**	0.5867*	0.8568**
0 to 6 h	0.6907*	0.6869*	0.6621*	0.4610	0.6419*

$* p < 0.05, ** p < 0.001$

0 to 1 h, 0 to 3 h and 0 to 6 h indicate CBV change over time from the endpoint of HI insult to 1, 3, and 6 hours after respectively.

The correlation coefficient between the changes in CBV and HIST scores at 1, 3, and 6 hours after resuscitation in the gray matter were 0.9016 (p<0.0001), 0.9127 (p<0.0001), and 0.6907 (p=0.0089), respectively. Those in the white matter were 0.8579 (p=0.0002), 0.8993 (p<0.0001), and 0.6869 (p=0.0095), respectively. Strong correlations were observed both in the gray and white matter. The correlation coefficients at 1, 3, and 6 hours in the hippocampus were 0.7347 (p=0.0042), 0.8437 (p=0.0003), and 0.6621

(p=0.0137), respectively. Those in the cerebellum were 0.5504 (p=0.0513), 0.5867 (p=0.0351), and 0.4610 (p=0.1129), respectively. The correlations were still significant in the hippocampus and cerebellum, albeit weaker than those in the gray and white matter.

The above results can be summarized as follows: 1) A correlation was noted between the degree of CBV change and HIST score, but the correlation was strong 1 and 3 hours after resuscitation and was slightly weaker after 6 hours. 2) Moreover, the strength of the correlation was as follows: gray and white matter > hippocampus > cerebellum.

Similarly, regarding the ScO_2 and HIST scores, no correlation was observed between the ScO_2 value and histological outcome in any of the parts (Figure 7 and Table 4).
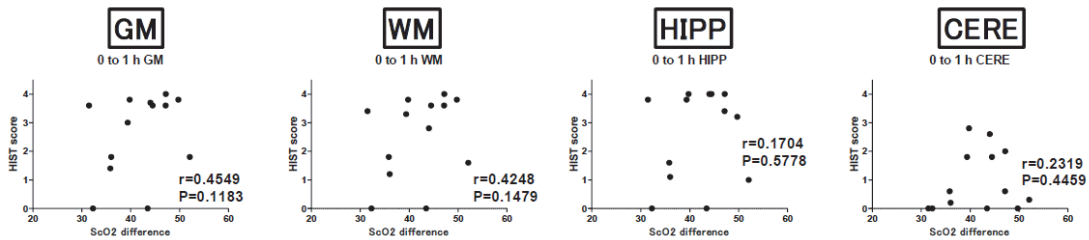
Figure 7

Figure 7. Relationship between the ScO_2 change and histopathology score from the end of insult to 1 h after for white matter, gray matter, the hippocampus, and cerebellum.
The histopathology score and changes in the ScO_2 in the gray matter, white matter, hippocampus and cerebellum at 1 hour after resuscitation are plotted along the vertical and horizontal axes. Spearman's rank correlation coefficient was used, and the correlation coefficient r was calculated. Unlike the CBV, no correlation was observed.

Table 4. Correlation coefficients between ScO_2 and HIST scores in different parts of the brain.

	GM	WM	HIPP	CERE	TOTAL
0 to 1 h	0.4549	0.4248	0.1704	0.2319	0.2920
0 to 3 h	0.3884	0.4028	0.1062	0.07264	0.2149
0 to 6 h	0.2441	0.3062	-0.06425	0.02794	0.08540

0 to 1 h, 0 to 3 h and 0 to 6 h indicate ScO_2 change over time from the endpoint of HI insult to 1,3, and 6 hours after respectively.

4. Discussion

In this study, we revealed that an increased CBV measured by TRS within 6 hours after HI insult indicates more severe hisitological brain damage. However, there was no association between the ScO_2 and histology in the matched time period.

4.1 Histological brain damage

There have been few reports on the severity or sites of impairment of cerebral circulation and brain tissue caused by HI insult. Williams et al.[25] studied EEG and tissue damage by changing the duration of ischemia due to cord occlusion in sheep fetuses. With the prolongation of ischemia, the damaged area of brain tissue widened, and the duration of suppression of EEG activities extended. The parasagittal cortex was the first site to be damaged. In addition, using sheep fetuses, damage to cerebellar tissue due to restriction of uterine blood flow [26] and hippocampal tissue damage due to 10-min cord occlusion [27] have been reported. In our present experiment, marked cerebral tissue damage was noted, especially in the gray and white matter, and the findings resembled the results of Williams et al.[25] with relatively stable and continuous HI insult.

4.2. Immediate measurement after HI insult

In the reported studies to date, the cerebral circulation in neonates with HIE has been evaluated mostly several to about 24 hours after birth and rarely from immediately to within several hours after resuscitation [6–8]. It has been reported on the basis of measurement of the CBF by positron emission tomography in the neonatal period (1 or more days, frequently about 1 week, after birth) that the outcomes were

unfavorable as the CBF after asphyxia was higher, and that a high CBF was related to the degree of failure of autoregulation [28,29].

In the measurement of cerebral oxygenation by NIRS, an increase in the ScO_2 after asphyxia has been shown to be associated with a poor outcome in both humans and animals. Kusaka et al.[5] measured the ScO_2 in newborn piglets exposed to hypoxic-ischemia, and reported that a high ScO_2 during 6-60 hours after the end of insult was correlated with a poor outcome. The ScO_2 showed a negative correlation with high-energy phosphate measured by phosphorus nuclear magnetic resonance spectroscopy (^{31}P-MRS). This increase in the ScO_2 was considered to indicate secondary energy failure. In humans, the accuracy of the prognosis has been reported to be improved by a combination of NIRS and aEEG, and Ancora et al. reported that the ScO_2 at 6, 12, and 24 hours after birth was significantly higher in a poor than a favorable outcome group. Toet et al. also reported on the basis of measurements from 12 to 48 hours after birth that the outcomes were unfavorable in the high- ScO_2 group and were related to abnormalities observed on aEEG [4,9]. In all of these studies, the measurements were performed 6 or more hours after HI insult, and the results are considered to reflect changes in cerebral oxygenation associated with secondary energy failure. While earlier measurement is important for determining the therapeutic strategy, there have been few reports suggesting the usefulness of assessment of the cerebral circulation/oxygen metabolism within 6 hours.

Nakamura et al. [21] reported a correlation between changes in the CBV and duration of LAEEG after HI insult. Therefore, the cerebral circulation may be evaluated earlier by measuring the change in the CBV rather than the duration of LAEEG. Iwata et al.[30] studied the relationship between the severity of HI insult and time until secondary

energy failure, that is, the latent phase, by magnetic resonance spectroscopy in newborn piglets, and reported that the latent phase was shorter and histological outcome was poorer with an increase in the severity of HI insult. However, the study showed the possibility of prolonging the latent phase and widening of the therapeutic window by the early introduction of therapeutic hypothermia. The duration of the latent phase depends on the severity of HI insult. In our experiment, the hemodynamics in an early stage of the latent phase were evaluated by measurement 6 hours after resuscitation.

Primary damage sustained early after birth may be estimated by evaluating changes in the cerebral circulation/oxygen metabolism very early after resuscitation. In a clinical setting, the severity or duration of HI in utero is not always clear. In our experiment (Figure 1), the CBV increased gradually from the beginning of hypoxic insult, reached a peak, and decreased gradually thereafter. In many of the newborn piglets in which the CBV was near the peak at the beginning of resuscitation, the CBV decreased rapidly after the beginning of resuscitation and the outcome was favorable. On the other hand, when resuscitation was initiated after the CBV increased to a peak and then decreased to a level near the baseline, the CBV increased again, and the survival state or histological outcome was extremely poor. This increase in the CBV is considered to be closely related to the severity of cerebral vasoparalysis and venous congestion due to heart failure.

The hemodynamics markedly change immediately after the beginning of resuscitation, and the evaluation of these changes by NIRS is considered to be very meaningful. In this study, we evaluated the hemodynamics after 1, 3, and 6 hours, and found that the increases in the CBV after 1 and 3 hours were more closely correlated with the severity of histological damage. Therefore, it is necessary to undertake further

study of the changes in the CBV within 3 hours, namely, from immediately after the beginning or during resuscitation. Monitoring very early changes in the CBV from immediately after birth of asphyxic neonates would help differentiate the severity of brain damage. As reports on changes in the cerebral circulation from immediately after birth and during resuscitation using NIRS have been increasing in number recently, vigilant and intensive evaluation using NIRS from immediately after birth is recommended for babies suspected of having been exposed to hypoxia in utero [31–34].

4.3 Limitations and future studies

In our newborn piglet model, data were obtained continuously during the hypoxic insult. Under these conditions, changes in the CBV from the end of HI insult to after 1 hour were most sensitively correlated with the histological outcome. In consideration of application to humans, however, there remain some clinical difficulties in consistently obtaining data from the moment of birth (from resuscitation) of an asphyxic neonate by attaching NIRS sensors at the bedside. Therefore, further analysis of detailed changes early after resuscitation based on animal experiments is necessary.

The relationships between an increase in the CBF and outcome that have been reported to date have mostly been those on secondary energy failure. However, the present study was focused on early changes in the CBV until 6 hours after birth, during which CBV changes are considered to reflect changes in the circulation due to hypoxia and the degree of vasoparalysis of the brain associated with primary damage. Since the time until secondary energy failure, namely, the latent phase, is considered to vary with the severity of insult, it is also considered significant to measure the CBV continuously from the latent phase to secondary energy failure beyond 6 hours. In addition, it is also

necessary to evaluate whether or not the CBV reflects the HIST score even with therapeutic intervention such as hypothermia and medication. Moreover, while the relationship with the CBF or cerebral metabolic rate of oxygen was not evaluated, these parameters are also important and must be evaluated in future studies. Furthermore, brain damage due to HI insult was evaluated in the present study, but separate evaluations regarding neonatal encephalopathy due to causes other than hypoxia-ischemia such as infection and hypoglycemia are also necessary.

5. Conclusion

An increase in the CBV immediately after HI insult predicts severe histological brain damage. Therefore, measurement of the CBV from immediately after resuscitation is useful for the neurological prognosis.

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