Hemodilutional anemia impairs neurologic outcome after cardiopulmonary bypass in a piglet model

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Objectives: The effect of hemodilution on neurologic outcome after cardiopulmonary bypass remains unclear. We studied the influences of hematocrit on cerebral oxygenation and neuropathologic outcome in a piglet model.

Methods: Eleven piglets (9.3 ± 1.1 kg) were randomized into 2 groups. Five piglets (group H) received a total blood prime resulting in a high hematocrit ($33.0\% \pm 2.3\%$), and 6 piglets (group L) received a crystalloid prime resulting in a low hematocrit ($14.0\% \pm 3.2\%$). Both groups underwent 90 minutes of moderate hypothermic cardiopulmonary bypass (28°C) with alpha-stat strategy. Cerebral oxygenation was monitored by near-infrared spectroscopy. Group L received a blood transfusion immediately after cardiopulmonary bypass to reach the postoperative target hematocrit of 30%. The brain was fixed in situ 6 hours after weaning from cardiopulmonary bypass, and a histologic score for neurologic injury was assessed.

Results: There were no significant differences in arterial blood gas analyses throughout the experiment between the groups. Mean arterial pressure, mixed venous oxygen saturation, and heart rate were significantly higher in group H compared with group L during hypothermia. Oxyhemoglobin and total hemoglobin signals detected by near-infrared spectroscopy were significantly lower in group L (analysis of variance, $P < .0001$), although the tissue oxygenation index was not different during cardiopulmonary bypass. Group L showed a poorer histologic score compared with group H ($P = .0071$).

Conclusions: Excessive hemodilution, such as a hematocrit of less than 15%, may be associated with a high incidence of neurologic injury. Further studies are required to determine the safety limits of hematocrit during pediatric cardiopulmonary bypass.

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Cardiopulmonary bypass (CPB) has contributed to the improvement of cardiovascular surgery. In this regard, CPB without using homologous blood transfusion has become common in adult cardiac surgery in recent years and has been applied in pediatric cardiac surgery. The advantage of this method is that it protects patients against the risk of graft-versus-host disease and viral infections such as hepatitis. On the other hand, extreme hemodilution reduces the oxygen-carrying capacity of blood and results in the impairments of systemic organs, particularly the brain. Recently, Jonas and colleagues¹ reported the importance of a higher hematocrit during CPB with deep hypothermic circulatory arrest. It is also reported that a lower hematocrit is associated with poorer neurologic outcome and renal failure in adult cardiac surgery with continuous CPB.² However, it is still unknown whether a higher hematocrit such as 30% is necessary for protecting the pediatric brain during continuous CPB without circulatory arrest. The aim of this study was to determine the effects of hemodilution under continuous CPB on cerebral function by using a neuromonitoring system such as near-infrared spectroscopy (NIRS) and histologic examination in a piglet model.

Abbreviations and Acronyms

CPB	= cardiopulmonary bypass
CytO ₂	= oxidized cytochrome a, a ₃
HbO ₂	= oxygenated hemoglobin
HbT	= total hemoglobin
HHb	= deoxygenated hemoglobin
NIRS	= near-infrared spectroscopy
TOI	= tissue oxygenation index

Materials and Methods**Experimental Preparation**

Eleven 4- or 5-week-old Yorkshire piglets, weighing 9.3 ± 1.1 kg, were sedated with intramuscular ketamine (20 mg/kg) and intubated with 5-mm cuffed endotracheal tubes. Each animal was ventilated at a peak inspiratory volume of 10 mL/kg at an inspired oxygen fraction of 0.21 and a rate of 12 to 18 breaths per minute by means of a volume-control ventilator (ACE-300; Acoma Corporation, Tokyo, Japan) to achieve a normal pH and arterial carbon dioxide tension. A pair of fiberoptic optodes for NIRS was placed on the head over the frontal lobes, with an interoptode distance of 4.0 cm. Anesthesia was induced with an intravenous bolus injection of midazolam (0.3 mg/kg) and pancuronium (0.5 mg/kg), and maintained by continuous inhalation of sevoflurane (0.8%-1.5%) and continuous infusion of midazolam (0.2 mg/kg/h) and pancuronium (0.2 mg/kg/h) throughout the experiment.

For the intraoperative monitoring and blood sampling, arterial and venous lines were placed in the left superficial femoral artery and right femoral vein, respectively. The right femoral artery was exposed for the CPB arterial cannula, and a right anterolateral thoracotomy was performed in the third intercostal space to expose the right atrium for venous cannulation. After systemic heparinization (300 IU/kg), an 8F arterial cannula (Medtronic Bio-Medics, Minneapolis, Minn) and a 28F venous cannula (Terumo Corporation, Tokyo, Japan) were inserted into the right femoral artery and right arterial appendage, respectively. All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council and published by the National Academy Press, revised in 1996.

Experimental Groups and Conditions

Piglets were randomized into 2 groups. Group H ($n = 5$) received a total fresh blood prime resulting in a high hematocrit of approximately 30%. Group L ($n = 6$) received crystalloid priming resulting in a low hematocrit of less than 20%. Operative conditions and intraoperative data were evaluated between the groups, and the relationship between the NIRS data and the neurologic outcome was examined comprehensively.

Cardiopulmonary Bypass Technique

The CPB circuit consisted of a roller-pump (Sarns 8000; Terumo Corporation, Tokyo, Japan) with a membrane oxygenator and sterile tubing (Capiiox Rx 05; Terumo Corporation, Tokyo, Japan).

The prime was determined according to the experimental protocol. Group H was primed with 1200 mL of whole blood. Group L was primed with 900 mL of crystalloid solution, 200 mL of hydroxyethylated starch, and 50 mL of mannitol. Methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), sodium bicarbonate (10 mL), midazolam (0.3 mg/kg), and pancuronium (0.5 mg/kg) were added to the prime. Full bypass flow was set at 100 mL/kg/min, and alpha-stat strategy was selected. CPB flow was maintained at 100 mL/kg/min except for the starting and the weaning of CPB in both groups. We did not correct the perfusion pressure because this CPB model followed a standard pediatric approach, which was a fixed-flow rate with no adjustment according to blood pressure. CPB was started, and the animals were perfused for 10 minutes at normothermic conditions (37°C). Ventilation was stopped after the establishment of CPB. Animals were then cooled to a nasopharyngeal temperature of 28°C for 10 minutes according to the experimental protocol. Both groups underwent continuous CPB for 60 minutes at 28°C. Before rewarming, methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), sodium bicarbonate (10 mL), and mannitol (0.5 g/kg) were administered into the pump. Animals were warmed to 37°C for 20 minutes. Ventilation was restarted 10 minutes before the weaning from CPB with an inspired oxygen fraction of 1.0. Animals were then weaned from CPB, and the arterial and atrial cannulas were removed. Protamine (5 mg/kg) was administered intravenously after the animal was hemodynamically stable. Group L received fresh whole blood from a donor pig, drawn on the day of the surgery, immediately after the weaning from CPB as required to increase the hematocrit to 30%. Minimum catecholamine was used at the time of weaning from CPB.

Postoperative Management

Animals remained sedated and paralyzed. They were mechanically ventilated and monitored continuously for 6 hours after the operation, and the brain was fixed in situ with 4 L of 4% formaldehyde solution. The histologic outcomes were assessed.

Data Collection**Blood Gas Analyses**

Arterial blood gas values, including electrolyte, glucose, and lactate concentrations, were measured at baseline, every 10 minutes during cooling and rewarming phase, every 30 minutes during moderate hypothermia, and every 60 minutes after weaning of CPB (i-STAT 300F; i-STAT Corporation, East Windsor, NJ). Blood gas values are presented for an electrode temperature of 37°C.

Near-infrared Spectroscopy

A pair of fiberoptic optodes was attached to the head of the animal with a probe holder after induction of anesthesia. The spacing of optodes was 4.0 cm in a coronal plane. These 2 optodes, a transmitter, and a receiver of laser light of near-infrared wavelength were connected to the NIRS (Niro300; Hamamatsu Photonics, Hamamatsu, Japan). This device calculates the relative concentration changes in oxygenated hemoglobin (HbO₂), deoxygenated hemoglobin

TABLE 1. Comparison of preoperative conditions of the two groups*

	Group H 30%	Group L 15%	P value†
Body weight (kg)	8.7 ± 0.6	9.2 ± 0.43	.49
pH	7.51 ± 0.04	7.52 ± 0.03	.72
Paco ₂ (mm Hg)	42.7 ± 3.1	38.9 ± 2.5	.35
Pao ₂ (mm Hg)	242 ± 26.4	217 ± 19.0	.46
Hematocrit (%)	29.6 ± 1.7	30.7 ± 1.3	.62
Osmolarity (mOsm/L)	280.19 ± 1.14	282.93 ± 1.74	.24
Lactate (mmol/L)	1.78 ± 0.70	1.64 ± 0.23	.84
Heart rate (beats/min)	108.8 ± 2.0	110 ± 4.9	.86
MAP (mm Hg)	67.8 ± 2.92	71.8 ± 4.22	.47
SvO ₂ (%)	83.6 ± 1.69	85.2 ± 1.25	.47
Nasopharyngeal temperature (°C)	34.2 ± 0.30	34.0 ± 0.44	.78

Paco₂, Arterial partial pressure of carbon dioxide; Pao₂, arterial partial pressure of oxygen; MAP, mean arterial pressure; SvO₂, venous oxygen saturation. *Mean ± standard error; 5 piglets in group H, 6 piglets in group L. †No significant differences were detected between the 2 groups according to the Student *t* test.

(HHb), and oxidized cytochrome a₃ (CytO₂), as well as tissue oxygenation index (TOI), which is calculated from the ratio of oxygenated to total hemoglobin (HbT). Data were recorded every 10 seconds after the induction of anesthesia and for 6 hours after weaning from CPB.

Histologic Assessment

Histologic assessment was performed as previously described.^{3,4} Histologic damage was rated in 10 sites (frontal lobe, temporal lobe, occipital lobe, thalamus, caudate nucleus, hippocampus, dentate gyrus, midbrain, pons, and medulla oblongata) with the following categoric scale (5 = cavitated lesions with necrosis, 4 = significant damage to neurons, 3 = large clusters of injured neurons, 2 = small clusters of damaged neurons, 1 = isolated neuronal damage, 0 = normal). Damage scores for all regions were summed and shown as the total histologic score. To avoid bias, all specimens were examined by 2 neuropathologists in a blinded fashion.

Statistical Analysis

All data were expressed in terms of the mean ± standard error. The Student *t* test was used to determine statistically significant differences between the 2 groups. Continuous data including hemodynamics and perfusion variables were compared between the 2 groups using repeated-measures of analysis of variance. Data were further compared by the Student *t* test if the analysis of variance was significant. Histologic scores were compared by the Mann-Whitney *U* test. Statistical analysis was performed using a statistical analysis software package (Stat-View version 5.0; Abacus Concepts, Berkeley, Calif).

TABLE 2. Changes in pH, arterial pressure of carbon dioxide, and arterial partial pressure of oxygen*

	Group H 30%	Group L 15%	P value†
pH			
Before operation	7.505 ± 0.098	7.523 ± 0.067	.7174
On CPB	7.509 ± 0.029	7.537 ± 0.034	.1711
End of CPB	7.506 ± 0.055	7.490 ± 0.047	.6313
cooling			
30 min	7.507 ± 0.064	7.509 ± 0.028	.9570
60 min	7.523 ± 0.069	7.525 ± 0.038	.9564
End of CPB	7.604 ± 0.073	7.650 ± 0.057	.2686
rewarming			
1 h	7.489 ± 0.068	7.544 ± 0.084	.2725
3 h	7.470 ± 0.025	7.497 ± 0.065	.4186
6 h	7.574 ± 0.073	7.555 ± 0.067	.6618
Paco ₂ (mm Hg)			
Before operation	42.70 ± 6.88	38.87 ± 6.05	.3509
On CPB	42.24 ± 3.70	37.47 ± 5.40	.1293
End of CPB	43.02 ± 4.09	41.78 ± 5.48	.6875
cooling			
30 min	43.24 ± 2.94	41.03 ± 2.40	.2025
60 min	40.56 ± 3.38	39.83 ± 2.37	.6850
End of CPB	34.94 ± 3.66	34.28 ± 3.67	.7739
rewarming			
1 h	40.06 ± 3.89	37.57 ± 4.17	.3356
3 h	45.06 ± 3.07	40.15 ± 4.52	.0699
6 h	38.38 ± 3.26	40.18 ± 1.86	.2772
Pao ₂ (mm Hg)			
Before operation	242.00 ± 59.06	217.50 ± 46.46	.4601
On CPB	358.80 ± 88.10	444.67 ± 52.41	.0753
End of CPB	608.80 ± 38.03	541.67 ± 72.52	.0963
cooling			
30 min	645.40 ± 47.19	594.83 ± 27.05	.0649
60 min	649.20 ± 42.25	601.83 ± 31.95	.0630
End of CPB	448.20 ± 41.03	451.33 ± 75.92	.9362
rewarming			
1 h	303.40 ± 91.12	308.17 ± 137.67	.9488
3 h	238.80 ± 67.19	240.50 ± 85.53	.9720
6 h	254.80 ± 21.21	252.83 ± 20.17	.8784

CPB, Cardiopulmonary bypass; Pao₂, arterial oxygen tension; Paco₂, arterial partial pressure of carbon dioxide. *Mean ± standard error; 5 piglets in group H, 6 piglets in group L. †Student *t* test detected differences.

Results

Experimental Conditions

There were no significant differences in baseline values between the 2 groups (Tables 1 and 2). During CPB, the hematocrit was significantly higher in group H relative to group L according to protocol (Table 3). Mean arterial pressure was significantly higher in group H compared with group L during the start of CPB, during the cooling, and in the moderate hypothermia phase (Table 4). In the moderate hypothermia phase of CPB, venous oxygen saturation was significantly higher in group H compared with group L (Table 5).

TABLE 3. Changes in hematocrit, lactate, and osmolarity*

	Group H 30%	Group L 15%	P value†
Hematocrit (%)			
Before operation	29.6 ± 3.8	30.7 ± 3.1	.621
On CPB	33.8 ± 1.9	14.7 ± 3.4	<.0001
End of CPB	33.0 ± 2.6	12.7 ± 2.9	<.0001
cooling			
30 min	33.0 ± 2.3	14.0 ± 3.2	<.0001
60 min	32.6 ± 2.1	15.0 ± 3.3	<.0001
End of CPB	31.0 ± 2.6	17.0 ± 2.9	<.0001
rewarming			
1 h	31.8 ± 1.8	28.8 ± 4.6	.2119
3 h	31.0 ± 4.6	27.7 ± 2.7	.1712
6 h	32.0 ± 2.6	33.5 ± 3.9	.4837
Lactate (mmol/L)			
Before operation	1.78 ± 1.57	1.64 ± 0.56	.8408
On CPB	2.06 ± 0.76	2.31 ± 2.00	.7984
End of CPB	3.03 ± 1.12	3.34 ± 2.04	.7704
cooling			
30 min	3.40 ± 1.38	3.43 ± 1.75	.9761
60 min	3.84 ± 1.47	2.67 ± 1.17	.1717
End of CPB	3.88 ± 1.29	3.61 ± 1.51	.7646
rewarming			
1 h	5.53 ± 2.70	6.35 ± 1.44	.5358
3 h	2.49 ± 1.08	3.59 ± 1.23	.1514
6 h	1.23 ± 0.44	1.83 ± 0.99	.2491
Osmolarity (mOsm/L)			
Before operation	280.19 ± 2.54	282.93 ± 4.27	.2415
On CPB	284.74 ± 2.43	286.34 ± 3.85	.4449
End of CPB	288.56 ± 3.89	288.80 ± 4.16	.9238
cooling			
30 min	290.72 ± 2.38	290.29 ± 5.06	.8651
60 min	291.94 ± 1.84	290.89 ± 3.39	.5500
End of CPB	288.10 ± 3.12	289.85 ± 4.26	.4648
rewarming			
1 h	290.39 ± 3.16	289.61 ± 3.75	.7211
3 h	285.33 ± 2.28	285.30 ± 3.16	.9927
6 h	286.58 ± 2.20	286.13 ± 2.99	.7854

CPB, Cardiopulmonary bypass. *Mean ± standard error; 5 piglets in group H, 6 piglets in group L. †Student *t* test detected differences.

Operative Results

One animal in group L demonstrated severe hypoxia after the weaning from CPB. All data in this animal were excluded from the analysis.

Near-infrared Spectroscopy

The HbO₂ signal in group H slightly increased after initiating CPB and remained at the same level during both the normothermic and moderate hypothermic phases. It gradually decreased during the rewarming phase. On the other hand, the HbO₂ signal in group L decreased and remained low throughout the CPB. After the weaning from CPB, this signal increased and reached baseline value. The HbO₂ signal was significantly lower in group L compared with group H during

TABLE 4. Changes in heart rate and mean arterial pressure*

	Group H 30%	Group L 15%	P value†
Heart rate (beats/min)			
Before operation	108.8 ± 4.4	109.8 ± 12.1	.8614
On CPB	130.2 ± 19.5	133.0 ± 20.2	.8216
End of CPB cooling	93.0 ± 9.0	95.3 ± 15.0	.7684
30 min	91.2 ± 11.1	98.7 ± 14.9	.3786
60 min	92.4 ± 12.8	97.5 ± 15.4	.5698
End of CPB	186.2 ± 9.5	164.8 ± 14.5	.0203
rewarming			
1 h	115.0 ± 23.5	131.8 ± 22.5	.2566
3 h	109.0 ± 19.7	100.7 ± 9.5	.3806
6 h	115.8 ± 6.2	107.7 ± 6.6	.0675
MAP (mm Hg)			
Before operation	67.8 ± 6.5	71.8 ± 10.3	.4711
On CPB	74.0 ± 2.6	47.7 ± 6.9	<.0001
End of CPB cooling	75.2 ± 2.7	51.5 ± 3.9	<.0001
30 min	75.8 ± 2.6	54.0 ± 2.4	<.0001
60 min	76.0 ± 3.1	55.2 ± 2.4	<.0001
End of CPB	71.2 ± 5.4	67.5 ± 14.4	.6029
rewarming			
1 h	73.0 ± 3.4	75.7 ± 8.1	.5124
3 h	80.0 ± 2.9	83.8 ± 7.9	.3318
6 h	85.8 ± 13.6	93.3 ± 8.2	.2838

CPB, Cardiopulmonary bypass; MAP, mean arterial pressure. *Mean ± standard error; 5 piglets in group H, 6 piglets in group L. †Student *t* test detected differences.

CPB ($P < .0001$). No significant difference was observed between the 2 groups after the weaning from CPB. The HHb signal in group H increased after initiating CPB and remained at the same level throughout CPB, whereas it remained at baseline value in group L. The signal in group H was significantly higher than that in group L during CPB ($P < .0001$). There was no significant difference between the 2 groups after CPB. The HbT signal showed almost the same pattern as the HbO₂ signal. The HbT signal was significantly lower in group L compared with group H during CPB ($P < .0001$). No significant difference was observed between the 2 groups after weaning from CPB. The CytO₂ signal remained at baseline value throughout CPB in group H. On the other hand, the signal in group L decreased after starting CPB and showed a significant difference throughout CPB compared with group H ($P < .0001$). There was no significant difference in TOI between the 2 groups ($P = .0705$). Some animals in both groups showed values of less than 50% during the rewarming phase (Figure 1).

Histologic Assessment

In group H, histologic damage was found in 2 sites (frontal lobe and thalamus). On the other hand, almost all the sites except for the dentate gyrus and brain stem showed histologic damage in group L. There were significant differences

TABLE 5. Changes in venous oxygen saturation and nasopharyngeal temperature*

	Group H 30%	Group L 15%	P value†
SvO₂ (%)			
Before operation	83.60 ± 3.78	85.17 ± 3.06	.4661
On CPB	88.20 ± 9.23	81.67 ± 9.75	.2865
End of CPB cooling	91.80 ± 1.10	87.67 ± 1.75	.0014
30 min	93.40 ± 1.82	89.33 ± 2.50	.0145
60 min	93.80 ± 1.30	90.83 ± 2.23	.0281
End of CPB rewarming	83.80 ± 8.23	78.17 ± 4.58	.1837
1 h	79.80 ± 5.02	77.67 ± 14.4	.7606
3 h	76.20 ± 5.76	79.00 ± 6.51	.4741
6 h	83.80 ± 6.22	80.17 ± 8.93	.4638
Nasopharyngeal temperature (°C)			
Before operation	34.16 ± 0.67	34.00 ± 1.07	.7792
On CPB	36.04 ± 0.43	35.68 ± 0.83	.4088
End of CPB cooling	28.26 ± 0.68	27.90 ± 0.73	.4248
30 min	27.92 ± 0.39	27.90 ± 0.42	.9370
60 min	27.86 ± 0.34	27.83 ± 0.46	.9166
End of CPB rewarming	36.58 ± 0.81	36.87 ± 0.65	.5297
1 h	34.24 ± 0.57	34.13 ± 0.83	.8138
3 h	35.28 ± 1.01	35.02 ± 0.91	.6731
6 h	35.50 ± 1.09	35.48 ± 1.09	.9804

CPB, Cardiopulmonary bypass; SvO₂, venous oxygen saturation. *Mean ± standard error; 5 piglets in group H, 6 piglets in group L. †Student *t* test detected differences.

in the hippocampus ($P = .0061$) and total histologic score ($P = .0071$) between the groups (Figure 2).

Discussion

The current study demonstrated that there is a higher risk of neurologic injury caused by low hematocrit during and after moderate hypothermic CPB without circulatory arrest. A low hematocrit of less than 15% is dangerous and should be avoided in pediatric cardiac surgery.

It has been controversial whether a hematocrit as high as 30% is necessary during CPB. The previous study states that a hematocrit of 15% to 20% is safe during CPB.⁵ In addition, it has been believed that a higher hematocrit under hypothermia results in slugging of red blood cells and can lead to inappropriate microcirculation. Recently, Dr. Jonas's group from Children's Hospital Boston reported that a hematocrit of 30% was better than 20% after CPB with deep hypothermic circulatory arrest in an experimental study^{4,6,7} and a randomized clinical trial.¹ Habib and colleagues² reported that a low hematocrit of less than 22% could lead to a higher possibility of multiple organ failure in 5000 adult patients with cardiac disease. However, these studies were performed in pediatric patients with hypothermic CPB consisting of circulatory arrest or reduced flow bypass at deep hypothermia or in adult patients with atherosclerosis. Therefore, there has been no evidence regarding optimal and safe

hematocrit during CPB without circulatory arrest and reduced flow at deep hypothermia in pediatric patients. The present study is the first to address this issue.

Histologic assessment should be considered the most reliable examination for brain ischemic damage. In the current study, damage in the frontal lobe and thalamus was seen in both groups. CPB itself may be a risk for cerebral ischemic damage. In general, the hippocampus is a more vulnerable region for ischemia. Group L revealed significantly poorer scores in the hippocampus compared with group H. This result suggests that straight hemodilution producing a hematocrit of less than 15% is associated with a higher risk of brain injury. A significant difference in the total histologic score strongly supports this hypothesis. In the meantime, we have performed histologic assessments of brain specimens that were fixed in situ at 6 hours after weaning from CPB. In a previous study it was demonstrated that ischemic cell death began as early as 6 hours after hypothermic circulatory arrest, reached its peak at 72 hours, and continued for at least 7 days.⁸ In the current study, the histologic score was significantly worse in group L compared with group H at 6 hours after CPB. If a specimen was fixed at 72 hours after CPB in the current study, it might be possible that the histologic score in group L may show more severe damage. In the future, we plan to perform a survival study including neurologic and behavioral evaluations, and histologic assessment of apoptosis induced by hemodilution. The future survival study could further elucidate the risk of hemodilution under CPB.

There was no significant difference in lactate between the 2 groups. The changes in lactate showed almost the same pattern between the groups. During CPB, lactate concentration was 3.0 mmol/L something in both groups, and the highest level was 5.53 ± 2.70 mmol/L in group H and 6.35 ± 1.44 mmol/L in group L at 1 hour after the operation. The levels had declined gradually and returned to the prebypass value 6 hours after the operation (Table 3). We speculated that there might not be significant circulatory failure, such as longer deep hypothermia and circulatory arrest even in group L. On the other hand, on the basis of the histology results in group L, we believe that the nadir hematocrit of 15% caused a disorder of tissue oxygenation leading to mild cerebral damages, not severe damages. However, the influence on the whole body was within limits, and there was no significant difference in lactate, which was derived from the systemic line, not the sagittal sinus.

NIRS^{9,10} has been recently used in cardiac surgery, and its use has been reported in both laboratory¹¹ and clinical studies.^{12,13} HbO₂, HHb, HbT, and CytO₂ signals detected by NIRS show the relative concentration changes of the baseline value, and the TOI reveals the tissue oxygenation that is derived from different parameters than the above-mentioned 4 parameters.¹⁴ In the current study, the NIRS device revealed a significantly higher value of HbO₂ and

Figure 1. Changes in NIRS data. A, HbO_2 signal. B, HHb signal. C, HbT signal. D, CytO_2 signal. E, TOI. ANOVA, Analysis of variance; BL, baseline; CPB, cardiopulmonary bypass; HbO_2 , oxygenated hemoglobin; HHb, deoxygenated hemoglobin; HbT, total hemoglobin; CytO_2 , oxidized cytochrome a, a_3 ; TOI, tissue oxygenation index; DPF, differential pathlength factor.

HbT in group H than in group L. The hematocrit in group H was approximately 33%, which was higher than the baseline value, whereas group L showed a low hematocrit of 10% to 15%. Differences of hematocrit could affect the HbO_2 and HbT values, and therefore this significant difference may not be important. Precisely, there was an increase of approximately 4% in hematocrit after initiating CPB in group H. This 4% increase may produce a higher level of HbO_2 and HbT signals in group H. On the other hand, the HHb value in group L remained at baseline level after initiating CPB when the hematocrit level was lower than the baseline value. The increase in HHb in group H also can be ex-

plained by the 4% increase in hematocrit. However, the HHb value in group L remained at baseline level after initiation of CPB, although the hematocrit level was lower than the baseline value. In general, when a cell is using enough oxygen, absolute deoxyhemoglobin value is theoretically almost zero. If zero value of the absolute deoxyhemoglobin is kept during CPB in group L, the HHb signal should be significantly lower than the baseline level because the hematocrit is almost half of the preoperative value. However, the result was different and the HHb signal remained at baseline level in group L. This indicates that the actual deoxyhemoglobin value (the absolute value) in-

Figure 2. A, Results of histologic scores. Mann-Whitney *U* test revealed differences in hippocampus and total histologic score. B, Histology from hippocampus, paraffin section, hematoxylin-eosin stain. a. Grade 0, showing no damage from group H ($\times 20$). b. Grade 3, showing hypoxic-ischemic injury from group L ($\times 20$). This is the most severely injured animal in this group. This would be scored as having a 3+ lesion. Note several neurons with hyper-eosinophilic cytoplasm and karyorrhectic nuclei (arrows).

creased after initiating CPB, suggesting that there was an increase in oxygen consumption. Therefore, CPB might lead to a lower oxygen condition in group L even during moderate hypothermia. This hypothesis is supported by the results of neuropathology in which the total histologic score in group L is worse than that in group H. On the other hand, in the current study, there was no significant difference in TOI between the 2 groups, although there were significant changes in the hematocrit level. However, some animals in each group showed a TOI of less than 50% during the rewarming phase, and neuropathology in the frontal lobe showed damages in both groups. Seven animals showed histologic damage in the frontal lobe: 3 in group H and 4 in group L. Among these, 5 piglets revealed a TOI of less than 50%. This is consistent with the previous report from Hagino and colleagues¹⁵ that a TOI of less than 55% is a strong predictor of neurologic injury. The NIRS device measures the tissue oxygenation of only the frontal lobe region. Therefore, a TOI of less than 50% might be associated with brain damage in selected regions (frontal lobe), and there might be a consistency between the NIRS data and

neuropathology. The difference between the total histologic score and TOI data may be explained by the above reasons. The alpha-stat strategy used in the current study may affect this result. The use of pH-stat strategy could avoid brain damage occurring in some regions even under the high hematocrit in group H.^{11,16} From this observation, we infer that it may be prudent to estimate the critical cerebral oxygenation during CPB with other parameters such as absolute cytochrome signal.^{17,18} Future studies are required to establish the absolute signal for detecting brain ischemia and the limits of safety of hematocrit during CPB.¹⁹ Nonetheless, cardiac surgeons should note that excessive hemodilution may have a higher incidence of postoperative neurologic damage even if there is no circulatory arrest. In addition, prospective, randomized clinical trials in combination with real-time monitoring should be planned to resolve this issue.^{20,21}

Limitations

There are 3 limitations to the current study: the effect of blood transfusion, the timing of blood transfusion, and the

perfusion pressure during CPB. Recently, Habib and colleagues²² reported that stored red blood cell transfusion during CPB increased the incidence of postoperative renal injury and hospital stays in adult patients undergoing isolated coronary artery bypass grafting. Also, Tsai and colleagues²³ showed that transfusion with stored red blood cells in a normovolemic anemic condition caused more malperfused and underoxygenated microvasculature that was not detectable at the systemic level compared with fresh whole-blood transfusion in an experimental study. In the current study, we used fresh whole blood from a donor piglet. It is unclear whether fresh whole blood has an influence on microvasculature in the brain. In regard to the timing of blood transfusion, there might be some difference in inflammation response. In the current study, group H was transfused at the start of CPB and group L was transfused after weaning from CPB. This might be associated with the difference in inflammatory response in the current study. Finally, this CPB model is a standard pediatric approach and reflects standard clinical practice. Therefore, the perfusion pressure was significantly lower in group L compared with group H during CPB because of a direct effect of viscosity secondary to lower hematocrit. The perfusion pressure is also important in oxygen delivery to the brain. In the current study, the perfusion pressure in group L was maintained at a mean arterial pressure of 50 mm Hg to 55 mm Hg during CPB. Therefore, there may be less correlation between the histologic score and perfusion pressure. The hematocrit during CPB may play a large part in brain oxygenation.

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