Intermittent pressure augmentation during retrograde cerebral perfusion under moderate hypothermia provides adequate neuroprotection: An experimental study

Mitsuhiro Kawata, MD, Shinichi Takamoto, MD, PhD, Kazuo Kitahori, MD, PhD, Hiroyuki Tsukihara, MD, Tetsuro Morota, MD, PhD, Minoru Ono, MD, PhD, Noboru Motomura, MD, PhD, Arata Murakami, MD, PhD, and Yoshihiro Suematsu, MD, PhD

Drs Kawata and Takamoto (left to right)

Objective: For cerebral protection during aortic surgery, we introduced a novel retrograde cerebral perfusion method with intermittent pressure augmentation. We then assessed whether this novel method provides benefits similar to those provided by antegrade selective cerebral perfusion.

Methods: Eighteen dogs were randomly divided into 3 groups: the RCP-INT group, intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg ($n = 6$); the ASCP group, antegrade selective cerebral perfusion at a flow rate of $10 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($n = 6$); and the sham group, no circulatory arrest ($n = 6$). Cooling (26°C) with cardiopulmonary bypass and 60 minutes of circulatory arrest were performed in the RCP-INT and ASCP groups. The levels of tau protein in the cerebrospinal fluid and the diameters of the retinal vessels were measured. The neurologic deficit scores and the histopathologic damage scores of the brains were determined.

Results: The total postoperative tau protein levels (calculated as the area under the curve) did not differ significantly between the RCP-INT and ASCP groups ($203 \pm 87 \text{ pg} \cdot \text{mL}^{-1} \cdot \text{h}$ vs $154 \pm 69 \text{ pg} \cdot \text{mL}^{-1} \cdot \text{h}$, $P = .95$). The retinal vessels were effectively dilated at an augmented pressure of 45 mm Hg in the RCP-INT group. The total neurologic deficit score (0 = normal, 500 = brain death) and histopathologic damage score (0 = normal, 40 = worst) were not significantly different between the RCP-INT and ASCP groups (neurologic deficit score: 75 ± 21 vs 70 ± 21 , $P = .98$; histopathologic damage score: 13.5 ± 1.5 vs 14.2 ± 1.3 , $P = .84$).

Conclusions: Intermittent augmented pressure dilated the cerebral vessels, allowing adequate blood supply without injuring the brain. This retrograde cerebral perfusion method provides adequate neuroprotection during moderate hypothermia.

From the Department of Cardiothoracic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

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Address for reprints: Mitsuhiro Kawata, MD, Department of Cardiothoracic Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan (E-mail: mkawata-ths@umin.ac.jp).

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We introduced a novel retrograde cerebral perfusion (RCP) method with intermittent pressure augmentation for cerebral protection during aortic surgery. The neuroprotective efficacy of this method, as compared with that of the conventional method, has been confirmed by our institution.¹ Intermittent augmented pressure effectively dilates cerebral vessels, allowing adequate blood supply to the brain while minimizing brain damage. In this study we examined whether this novel method could provide similar clinical benefits to those provided by antegrade selective cerebral perfusion (ASCP) during moderate hypothermia.

Materials and Methods

Animal Care

This study was approved by the Animal Care and Use Committee of the University of Tokyo. All the animals were acclimatized in the Section of Animal Research of the Center for Disease

Abbreviations and Acronyms

- ASCP = antegrade selective cerebral perfusion
- AUC = area under the curve
- CPB = cardiopulmonary bypass
- CSF = cerebrospinal fluid
- HDS = histopathologic damage score
- NDS = neurologic deficit score
- RCP = retrograde cerebral perfusion

Biology and Integrative Medicine. All received humane care in compliance with the 1996 “Guide for the care and use of laboratory animals” (1996 Institute for Laboratory Animal Research).

Experimental Groups

Eighteen adult mongrel dogs, each weighing 23 to 32.1 kg (mean, 28.7 kg) were randomly divided into the following 3 groups: the RCP-INT group, RCP through both maxillary veins at a baseline pressure of 15 mm Hg with intermittent pressure augmentation every 30 seconds to 45 mm Hg by only increasing the flow of the pump manually (n = 6); the ASCP group, ASCP at a flow rate of 10 mL · kg⁻¹ · min⁻¹ through an arterial cannula in the ascending aorta after clamping of the proximal ascending aorta, left subclavian artery, and descending aorta (n = 6); and the sham group, no circulatory arrest (n = 6).

Animal Preparation

All animals were premedicated with ketamine hydrochloride (10 mg/kg administered intramuscularly) and anesthetized and maintained with sodium pentobarbital throughout the operation. Support with a pressure-controlled ventilator (Bird Mark-7 respirator; Bird Products Corp, Viasys Healthcare Inc, Palm Springs, Calif) was started at 100% oxygen after endotracheal intubation. A partial laminectomy at the level of the first lumbar vertebra was performed, and a 20-gauge catheter was inserted into the external cerebrospinal fluid (CSF) space toward the cranial side to allow continuous pressure monitoring of the CSF and sampling at several time points. The femoral artery and the external jugular vein were cannulated with 20-gauge catheters for blood sampling, and the arterial and central venous pressures were monitored continuously. In the RCP-INT group two 16-gauge cannulas were inserted into each of the maxillary veins of either side; these cannulas were used only during RCP. Blood samples were analyzed after correction for the animal’s body temperature. Oxygen saturation, pH, oxygen tension, carbon dioxide tension, base excess, carbonic acid, electrolytes, and hemoglobin levels were measured with a blood gas analyzer (ABL505, Radiometer Medical Aps, Copenhagen, Denmark) and a hemoglobin and oxygen saturation analyzer (OMS2 Hemoximeter, Radiometer Medical Aps). The core temperature of the animals was monitored by using probes in the esophagus and rectum.

Experimental Protocol

Before systemic heparinization, 400 mL of blood was removed for hemodilutional autologous transfusion. A median sternotomy was performed. After systemic heparinization (300 IU/kg), a 16F arterial cannula (Medtronic, Inc, Minneapolis, Minn) was inserted in

TABLE 1. A. Criteria for neurologic deficit score

A. Central nerve function			
Variable	Normal	Weak	Absent
Light reflex	0	5	20
Lid reflex	0	5	20
Corneal reflex	0	5	20
Auditory reflex	0	5	20
Carinal reflex	0	5	20
B. Respiration condition			
Normal	0		
Hyperventilation	25		
Abnormal	50		
Apnea	100		
C. Motor and sensory function			
Variable	Normal	Weak	Absent
Movement of limbs	0	20	50
Motor response to pain	0	20	50
D. Level of consciousness			
Normal	0		
Cloudy	30		
Delirium	45		
Stupor	60		
Coma	100		
E. Abnormality			
No convulsion	0		
Temporal convulsion	50		
Serial recurrent convulsion	100		

Total = sum of all sections. Best possible total neurologic deficit score = 0; worst possible total neurologic deficit score = 500.

the ascending aorta, and a 36F venous single cannula (Terumo Co, Ltd, Tokyo, Japan) was inserted in the right atrium. Extracorporeal circulation was performed with a membrane oxygenator (Capiiox SX, Terumo Co, Ltd) and an extracorporeal pump (MHS-15-IV, MERA; Senko Medical Instrument Mfg, Co, Ltd, Tokyo, Japan) containing a circuit primed with a hemodilute solution of 800 mL of lactated Ringer’s solution, 50 mL of 20% human albumin, 40 mL of sodium bicarbonate, 200 mL of mannitol, and 5000 IU of heparin. Cardiopulmonary bypass (CPB) was established at a flow rate of 100 mL · kg⁻¹ · min⁻¹, with the flow adjusted to maintain a mixed venous oxygen saturation of approximately 75%. A 14-gauge catheter was inserted into the left ventricle from the apex to permit decompression of the left ventricle during CPB. The animals were cooled to 26°C (moderate hypothermia) with a heat exchanger. The pH was maintained at 7.40 by means of pH-stat principles, with an arterial PaCO₂ of 35 to 40 mm Hg, corrected for body temperature. Cardiac arrest was induced by using cold cardioplegic solution after crossclamping of the ascending aorta. Then the animals (except those in the sham group) underwent 60 minutes of circulatory arrest. During the circulatory arrest, brain protection procedures were carried out according to the group definition, as mentioned previously. In the RCP-INT group the arterial cannula in the ascending aorta was opened to maintain the common carotid arterial pressure at atmospheric pressure.

After the period of circulatory arrest, CPB was reestablished. Cardioversion was performed, if necessary, to resume the sinus

TABLE 1. B. Neurologic deficit score after the operation

	RCP-INT	ASCP	Sham	P value
A. Central nerve function	2.5 ± 1.1	3.3 ± 1.1	3.3 ± 1.1	.82
B. Respiration condition	8.3 ± 5.3	8.3 ± 5.3	8.3 ± 5.3	1.00
C. Motor and sensory function	20 ± 5	13 ± 4	13 ± 4	.51
D. Level of consciousness	20 ± 6	20 ± 6	15 ± 7	.82
E. Abnormality	25 ± 11	25 ± 11	17 ± 11	.83
Total	75 ± 21	70 ± 21	57 ± 23	.83

Best possible total neurologic deficit score = 0; worst possible total neurologic deficit score = 500. *RCP-INT*, Intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg; *ASCP*, antegrade selective cerebral perfusion at a flow rate of 10 mL · kg⁻¹ · min⁻¹.

rhythm at approximately 34°C, and mechanical ventilation was restarted. All animals were then rewarmed to 37°C by using a heat exchanger and an infrared heater and were slowly weaned from CPB. After hemodynamic measurements, protamine was administered, and the CPB solution was removed. Autologous blood (collected before the operation) was then transfused. Finally, all the wounds were closed. The animals were kept connected to the ventilator throughout the procedure.

Observation of Retinal Vessels

Eye drops of atropine sulfate were applied preoperatively into the eyes of the animals. A fundus camera (Genesis; Kowa Co, Ltd, Nagoya, Japan) was used to take pictures of the retinas, including the retinal vessels. The diameters of the 3 major retinal arteries and those of the veins were measured at the edge of the retinal macula and were expressed as a ratio relative to the corresponding preoperative (36°C) control values.

Neurologic Assessment

A postoperative neurologic assessment was performed in each animal by an independent observer. The modified neurologic deficit score (NDS) in dogs²⁻⁴ was used to evaluate neurologic deficits. The NDS evaluates 5 general components of the neurologic examination (central nerve function, respiration, motor and sensory function, level of consciousness, and behavior), with a score of 0 to 100 assigned to each category. A total score of 500 indicates the worst possible neurologic damage, whereas a score of 0 is normal. All the animals were closely evaluated for seizure activity. In this study, the “behavior” component of the NDS was modified to “abnormality” to enable the early postoperative condition to be evaluated (Table 1, A).

Tau Protein Levels in the CSF

The CSF was sampled at several time points: before the operation, during CPB (rewarming), and 3, 6, and 12 hours after the operation. The samples were centrifuged and stored at -80°C until analysis. The tau protein concentration, a marker of neuronal damage, was then measured with a commercially available enzyme-linked immunosorbent assay kit (Fino Scholar hTAU; Nipro, Osaka, Japan). The postoperative total tau protein level in each dog was calculated as the area under the curve (AUC) by using a trapezoidal method.

Histopathologic Examination

At 12 hours after the operation, the experiment was terminated, and the histopathologic studies were performed. The brains were quickly harvested immediately after the induction of cardiac arrest and after achievement of adequate anesthesia. The brains were then fixed with 7% buffered formaldehyde solution. Coronal sections (5-mm thick) of the brain were examined for gross lesions. Sections of interest were embedded in paraffin, cut to a thickness of 10 μm, stained with hematoxylin and eosin, and examined with light microscopy. Five anatomic areas (frontal lobe, parietal lobe, thalamus, hippocampus CA-1, and cerebellum) were examined by a pathologist who was unaware of the experimental groups. During the early period after the onset of hypoxic ischemic injury, the minimum criteria for the diagnosis of ischemic neuronal damage include mild cytoplasmic eosinophilia, shrunken neurons with scalloping of the margins, and nuclear changes consisting of coarse nuclear chromatin or pyknosis.⁵⁻⁸ A modified histopathologic damage score (HDS)² was used to determine the histopathologic damage. The HDS was defined as follows: no damaged neurons (0), minimal (2), mild (4), moderate (6), or severe (8).

Statistical Analysis

All data were presented as the mean ± standard error of the mean. Data were assessed by using a 1-way analysis of variance for comparisons among the groups, followed by a post-hoc Dunnett test. The Spearman rank order correlation coefficient was used to correlate the 12-hour postoperative NDS and the total tau protein levels, as well as the NDS and HDSs and the total tau protein levels and HDS. All statistics were computed with the JMP analysis program, version 5.1 (SAS Institute Inc, Cary, NC).

Results

Physiologic Variables

There were no significant differences in the preoperative physiologic variables among the groups. The CSF pressure varied with the perfusion pressure of RCP. During RCP at the augmented pressure of 45 mm Hg, the CSF pressure became significantly higher in the RCP-INT group (32 ± 4.0 cm CSF) than in the ASCP group (14 ± 1.9 cm CSF; *P* < .01, Dunnett post-hoc test). During RCP at the baseline pressure of 15 mm Hg, the CSF pressure was not signifi-

Table 2. Physiologic variables

	RCP-INT	ASCP	Sham	P value
Body weight (kg)	28 ± 1	28 ± 1	29 ± 1	.40
Sex (female/male ratio)	3/3	3/3	3/3	1.00
MAP (mm Hg)				
Preop	121 ± 12	128 ± 6	133 ± 9	.63
On CPB	67 ± 5	69 ± 5	69 ± 5	.85
Post3h	99 ± 5	116 ± 6	103 ± 7	.14
Post6h	98 ± 3	122 ± 12	112 ± 11	.21
Post12h	81 ± 9	109 ± 9	98 ± 15	.25
CSF pressure (cm CSF)				
Preop	17 ± 0.6	16 ± 0.8	17 ± 0.9	.24
During cerebral perfusion	19 ± 0.7 (15 mm Hg)	15 ± 1.6	19 ± 1.0	.06
	32 ± 4.0 (45 mm Hg)			.0007*
Post3h	23 ± 2.6	24 ± 5.4	25 ± 3.9	.94
Post6h	24 ± 5.0	25 ± 4.5	27 ± 4.3	.84
Post12h	23 ± 2.8	23 ± 2.3	26 ± 4.5	.83
Pao ₂ (mm Hg)				
Preop	349 ± 24	346 ± 23	429 ± 56	.24
On CPB	492 ± 30	533 ± 37	438 ± 29	.20
Post3h	304 ± 95	323 ± 67	379 ± 91	.82
Post6h	390 ± 88	373 ± 82	385 ± 79	.98
Post12h	400 ± 79	343 ± 87	378 ± 68	.87
Paco ₂ (mm Hg)				
Preop	34 ± 1	30 ± 2	34 ± 2	.29
On CPB	42 ± 4	40 ± 2	38 ± 2	.72
Post3h	40 ± 5	40 ± 6	48 ± 3	.28
Post6h	40 ± 5	37 ± 6	50 ± 3	.16
Post12h	38 ± 3	37 ± 6	47 ± 6	.3
Arterial pH				
Preop	7.38 ± 0.04	7.40 ± 0.02	7.43 ± 0.02	.54
On CPB	7.24 ± 0.06	7.28 ± 0.04	7.37 ± 0.03	.11
Post3h	7.32 ± 0.04	7.42 ± 0.07	7.32 ± 0.01	.26
Post6h	7.36 ± 0.05	7.44 ± 0.06	7.29 ± 0.02	.13
Post12h	7.36 ± 0.03	7.41 ± 0.06	7.31 ± 0.04	.33
Svo ₂ (%)				
Preop	82 ± 4	86 ± 4	87 ± 5	.74
On CPB	86 ± 5	86 ± 3	85 ± 5	.97
Post3h	76 ± 8	71 ± 5	71 ± 6	.81
Post6h	72 ± 7	65 ± 7	68 ± 7	.80
Post12h	69 ± 10	72 ± 5	69 ± 6	.94
Hemoglobin (g/dL)				
Preop	13.0 ± 0.4	13.5 ± 1.0	12.0 ± 0.8	.31
On CPB	5.5 ± 0.3	5.5 ± 0.3	5.0 ± 0.3	.33
Post3h	12.0 ± 0.4	12.3 ± 0.7	11.0 ± 0.7	.20
Post6h	11.0 ± 0.9	11.0 ± 0.9	11.3 ± 0.9	.86
Post12h	11.4 ± 1.0	10.8 ± 0.9	11.7 ± 0.9	.81

RCP-INT, Intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg; ASCP, antegrade selective cerebral perfusion at a flow rate of 10 mL · kg⁻¹ · min⁻¹; MAP, mean arterial pressure; preop, preoperative; CPB, cardiopulmonary bypass; post3h, 3 hours after the operation; post6h, 6 hours after the operation; post12h, 12 hours after the operation; CSF, cerebrospinal fluid; Svo₂, mixed venous saturation of oxygen. *P < .05.

cantly different among the groups ($P = .06$), although the ASCP group tended to show lower CSF pressures. No other parameters were significantly different among the groups (Table 2).

Retinal Vessels

In the RCP-INT group the retinal vessels were effectively dilated at an augmented pressure of 45 mm Hg (arteries, 107% ± 3% of control, $P = .041$; veins, 114% ± 3% of

Figure 1. Fundus photography. Panels A through D are from the intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg (RCP-INT) group. Panels E and F are from the antegrade selective cerebral perfusion at a flow rate of $10 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (ASCP) group. Panels G and H are from the sham group. A, E, and G, Before CPB at 36°C; B, 15 mm Hg of RCP at 26°C; C, RCP at 25 mm Hg at 26°C; D, RCP at 45 mm Hg at 26°C; F, during antegrade selective cerebral perfusion at 26°C; and H, during cardiopulmonary bypass at 26°C without circulatory arrest.

Figure 2. Diameters of the retinal arteries and veins in the intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg (RCP-INT), antegrade selective cerebral perfusion at a flow rate of $10 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (ASCP), and sham groups. Values for the retinal arteries and veins are expressed as ratios relative to the corresponding preoperative (36°C) control values.

control, $P < .01$). In the RCP-INT (perfusion pressure of 15 mm Hg), ASCP, and sham groups the retinal vessels at 26°C were smaller than the corresponding preoperative (36°C) vessels (Figures 1 and 2).

Neurologic Assessment

The final total NDS at 12 hours after the operation did not differ significantly among the groups (RCP-INT, 75 ± 21 ; ASCP, 70 ± 21 ; sham, 57 ± 23 ; $P = .83$; ASCP vs RCP-INT, $P = .98$, Dunnett post-hoc test). For each neurologic component, no significant differences were observed among the groups (Table 1, B).

Tau Protein Levels in the CSF

No significant differences in the tau protein concentrations before the operation were seen among the groups (RCP-INT,

$44 \pm 9 \text{ pg/mL}$; ASCP, $19 \pm 9 \text{ pg/mL}$; sham, $25 \pm 13 \text{ pg/mL}$; $P = .23$). The tau protein levels did not differ significantly among the groups during CPB (RCP-INT, $11 \pm 5 \text{ pg/mL}$; ASCP, $2 \pm 2 \text{ pg/mL}$; sham, $19 \pm 9 \text{ pg/mL}$; $P = .18$) or at 3 hours (RCP-INT, $2 \pm 2 \text{ pg/mL}$; ASCP, $10 \pm 4 \text{ pg/mL}$; sham, $6 \pm 3 \text{ pg/mL}$; $P = .33$), 6 hours (RCP-INT, $9 \pm 5 \text{ pg/mL}$; ASCP, $19 \pm 12 \text{ pg/mL}$; sham, $1 \pm 1 \text{ pg/mL}$; $P = .24$), and 12 hours (RCP-INT, $46 \pm 23 \text{ pg/mL}$; ASCP, $13 \pm 10 \text{ pg/mL}$; sham, $67 \pm 62 \text{ pg/mL}$; $P = .62$) after the operation. The total tau protein levels after the operation (AUC value over 12 hours) also were not significantly different among the groups (RCP-INT, $203 \pm 87 \text{ pg} \cdot \text{mL}^{-1} \cdot \text{h}$; ASCP, $154 \pm 69 \text{ pg} \cdot \text{mL}^{-1} \cdot \text{h}$; sham, $250 \pm 197 \text{ pg} \cdot \text{mL}^{-1} \cdot \text{h}$; $P = .87$; ASCP vs RCP-INT, $P = .95$, Dunnett post-hoc test; Figure 3). A significant correlation between the final total NDS and the

Figure 3. Tau protein levels in the cerebrospinal fluid (CSF). *RCP-INT*, Intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg; *ASCP*, antegrade selective cerebral perfusion at a flow rate of 10 mL · kg⁻¹ · min⁻¹; *preop*, preoperative; *post6h*, 6 hours after the operation; *post12h*, 12 hours after the operation. No significant differences in the tau protein levels were seen among the groups.

total tau protein level was seen (Spearman correlation coefficient = 0.8349, $P < .0001$).

Histopathologic Examination

No macroscopic gross lesions were observed in any of the brain specimens. The total HDS did not differ significantly among the groups (*RCP-INT*, 14 ± 1.3 ; *ASCP*, 13 ± 1.5 ; *sham*, 13 ± 2.1 ; $P = .77$; *ASCP* vs *RCP-INT*, $P = .84$, Dunnett post-hoc test). The regional HDSs for the 5 selected anatomic areas did not differ significantly among the groups (Figure 4). A significant correlation was observed between

the final total NDS and the total HDS (Spearman correlation coefficient = 0.7498, $P = .0003$). A significant correlation between the total tau protein levels and the total HDS was also observed (Spearman correlation coefficient = 0.7973, $P < .0001$). Samples from all the groups showed only minimal evidence of cellular change and no evidence of either cerebral edema or hemorrhage (Figure 5).

Discussion

Higher RCP pressure methods^{9,10} and intermittent RCP methods^{11,12} have also been reported, with good outcomes

Figure 4. Histopathologic damage score. *RCP-INT*, Intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg; *ASCP*, antegrade selective cerebral perfusion at a flow rate of 10 mL · kg⁻¹ · min⁻¹; *FL*, frontal lobe; *PL*, parietal lobe; *THA*, thalamus; *HIPCA1*, hippocampus CA1 area; *CER*, cerebellum; *Total*, total histopathologic damage score. No significant differences in the histopathologic damage score were seen among the groups.

Figure 5. Histopathologic examination. Panels A, C, and E are sections of the hippocampus CA1 area. (Hematoxylin and eosin; original magnification 200 $\times$.) Panels B, D, and F are sections of the cerebellum. (Hematoxylin and eosin, original magnification 200 $\times$). Panels A and B are from the intermittent-retrograde cerebral perfusion at 15 mm Hg with intermittent pressure augmentation to 45 mm Hg (RCP-INT) group. Panels C and D are from the antegrade selective cerebral perfusion at a flow rate of 10 mL $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ (ASCP) group. Panels E and F are from the sham group. All of the sections show only minimal evidence of cellular change and no evidence of either cerebral edema or hemorrhage.

and no significant complications. Li and colleagues⁹ reported that higher RCP pressures (34-40 mm Hg) amplified cerebral tissue perfusion and tissue oxygenation, without increasing tissue edema. Estrera and associates¹⁰ showed that the mean RCP peak pressure for identifying cerebral blood flow was 31.8 ± 9.7 mm Hg. Although they examined the application of high RCP pressures, they did not observe evidence of increased cerebral edema. A high RCP pressure of approximately 40 to 50 mm Hg is thought to be needed to overcome the threshold for the dilation of cerebral microvessels during hypothermia. By applying this high pressure intermittently, we hoped to prevent the cerebral edema that is caused by a continuous high RCP pressure. The present study showed no evidence of increased cerebral edema in the RCP-INT group.

The cerebral blood flow autoregulation is not significant under conditions of hypothermia. A body temperature of 26°C, cardiac arrest, and circulatory arrest to half of the body are nonphysiologic situations for living animals. Therefore we think that control of artificial cerebral perfusion is needed.

The blood returned to the aortic arch through the cerebral vessels in the RCP-INT group was obviously venous dark blood, in which the oxygen saturation was significantly lower than that in the perfused oxygenated blood. The returned blood volume to the aortic arch in the RCP-INT group was about 60% to 80% of the volume of the perfused oxygenated blood.

Observation of the retinal vessels is a direct, sensitive, and noninvasive method for observing cerebral blood flow and assessing the effects of cerebral perfusion.^{1,13-15} We concluded that dilatation of the retinal vessels in the RCP-INT group was reflective of adequate cerebral flow because we also observed the blood passing through the retinal veins and capillary vessels to the retinal arteries using a fundus camera. A similar conclusion was also drawn by Ono and coworkers¹⁴ in conventional RCP. We monitored not only the vascular dilatation but also observed the blood passing through the vessels. During hypothermia, all the groups showed vasoconstriction of the retinal vessels (RCP-INT [15 mm Hg], ASCP, and sham). However, during RCP-INT (25 mm Hg), no significant differences were observed from the corresponding preoperative condition (36°C). We thought that this result was due to the gradual opening up of the blood vessels during the intermittent pressure augmentation to 45 mm Hg. We confirmed similar vasoconstriction of the retinal blood vessels in the conventional RCP (fixed perfusion pressure of 25 mm Hg) group as in the ASCP and sham groups. On the other hand, significant vascular opening was observed in the RCP-INT (45 mm Hg) group compared with that seen in the other groups.

Only minimal evidence of acute ischemic neuronal cell changes was seen in all the groups. This suggests that RCP-INT and ASCP during moderate hypothermia provided a similar extent of protection against acute ischemic neuronal cell changes. During RCP at an augmented pressure of 45 mm Hg, the CSF pressure was significantly higher in the RCP-INT group than in the ASCP group. However, the CSF pressure was not significantly different among the groups when a baseline pressure of 15 mm Hg was applied during RCP. Therefore cerebral edema was not observed in the RCP-INT group.

Tau protein level in the CSF is considered a good biomarker of neuronal damage.¹⁶⁻²⁰ We believe that the results of tau protein levels would have been the same even after 24 or 48 hours because the tau protein levels in the CSF in individual cases did not show any steady increase in all groups. Some cases remained steady, and other cases

showed a peak out pattern. Therefore we compared the total tau protein levels by using AUC. The total tau protein levels showed no significant differences among the groups.

Although some authors have questioned the validity of the neurologic protection provided by RCP,^{6,21} this study showed that our modification of the RCP technique produced a satisfactory neuroprotective outcome equivalent to that provided by ASCP.

Study Limitations

If the dogs had been observed for a longer period of time, such as 1 week or more, a greater difference in functional outcome might have been seen. However, allowing long-term survival of brain-damaged and laminectomized animals is neither feasible nor humane. Therefore we decided to limit our postoperative observations to a 12-hour period. We used moderate hypothermia of 26°C for 60 minutes in this study. Additional studies under different conditions are necessary.

Conclusion

This study showed that intermittent pressure augmentation during RCP efficiently dilated the cerebral vessels, allowing an adequate blood supply without injuring the brain. This RCP-INT during moderate hypothermia provides adequate neuroprotection equivalent to that provided by ASCP.

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