Erythropoietin protects the central nervous system during prolonged hypothermic circulatory arrest: An experimental study in a canine model

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Objective: Current data suggest that erythropoietin protects the brain and the spinal cord from ischemic and traumatic injury. In this study, we determined whether erythropoietin protects the central nervous system during prolonged hypothermic circulatory arrest in an experimental canine model.

Methods: Ten adult beagle dogs were randomly and intravenously injected with either 5000 U/kg recombinant human erythropoietin or normal saline. Each dog was then subjected to a cardiopulmonary bypass and 120 minutes of deep hypothermic circulatory arrest (18°C). The level of tau proteins in the cerebrospinal fluid, a modified marker of neurologic deficit in dogs, and the histopathologic characteristics of the brains and spinal cords were then examined.

Results: The level of tau proteins was significantly lower in the erythropoietin-treated group than in the untreated group at 6 hours (20 ± 12 vs 144 ± 54 pg/mL; $P = .036$) and 12 hours (64 ± 35 vs 478 ± 103 pg/mL; $P = .01$) after the operation. The total Neurologic Deficit Score was 59 ± 31 (0, normal; 500, brain death) in the erythropoietin-treated group, compared with 376 ± 30 in the untreated group ($P = .0117$). Histopathologic examination revealed that ischemic neuronal changes and apoptosis in the hippocampus CA1 were significantly lower in the erythropoietin-treated group ($P < .01$ and $P = .028$, respectively).

Conclusions: This study showed that erythropoietin protected the central nervous system during prolonged hypothermic circulatory arrest, partly by preventing both necrosis (ischemic neuronal changes) and apoptosis.

Erythropoietin (EPO) is a glycoprotein that was first characterized as a kidney cytokine that regulates hematopoiesis, or the production of blood cells. EPO is also produced in the brain after oxidative or nitrosative stress.^{1,2} Current data suggest that EPO protects the brain and spinal cord from ischemic and traumatic injury.^{3,4} We hypothesized that EPO might extend the duration of safe circulatory arrest. In this study, we determined whether EPO protects the central nervous system during prolonged hypothermic circulatory arrest in an experimental canine model.

Abbreviations and Acronyms

CPB	= cardiopulmonary bypass
CSF	= cerebrospinal fluid
EPO	= erythropoietin
HDS	= Histopathologic Damage Score
NDS	= Neurologic Deficit Score
TUNEL	= deoxyuridine-5'-triphosphate biotin nick end labeling

Materials and Methods**Animal Care**

This study was approved by the Animal Care and Use Committee of the University of Tokyo. All animals were acclimatized in the Section of Animal Research of the Center for Disease Biology and Integrative Medicine. All animals received humane care in compliance with the 1996 "Guide for the Care and Use of Laboratory Animals" (Institute for Laboratory Animal Research).

Experimental Groups

Ten adult beagle dogs weighing 10 to 13 kg (mean, 11.5 kg) and 9 to 20 months of age (mean, 15.3 months) were randomly assigned to receive an intravenous injection of either EPO (ESPO; epoetin alpha genetic recombination; 5000 U/kg body weight; Pharmaceutical Division of Kirin Brewery Co Ltd, Tokyo, Japan) (EPO group) or a saline placebo (control group) directly before undergoing a sternotomy.

Experimental Protocol

All animals were premedicated with ketamine hydrochloride (10 mg/kg intramuscularly), and anesthesia was induced and maintained with sodium pentobarbital throughout the operation. Support with a pressure-controlled ventilator (Bird Mark-7 respirator; Bird Products Corporation, Viasys Healthcare Inc, Conshohocken, Pa) was started on 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 space containing cerebrospinal fluid (CSF) 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. Blood samples were analyzed after correction for body temperature, pH, oxygen tension, carbon dioxide tension, base excess, carbonic acid, electrolytes, hemoglobin, and oxygen saturation by using 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 was monitored in the esophagus and rectum.

Hypothermic Circulatory Arrest Using Cardiopulmonary Bypass

Before systemic heparinization, 200 mL of blood was removed for hemodilutional autologous transfusion. A median sternotomy was performed. After systemic heparinization (300 IU/kg), a 10F arte-

TABLE 1. Criteria for Neurologic Deficit Score

Variable	Normal	Weak	Absent
Central nerve function			
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
Respiration condition			
Normal			0
Hyperventilation			25
Abnormal			50
Apnea			100
Motor and sensory function			
Movement of limbs	0	20	50
Motor response to pain	0	20	50
Level of consciousness			
Normal			0
Cloudy			30
Delirium			45
Stupor			60
Coma			100
Abnormality			
No convulsion			0
Temporal convulsion			50
Serial recurrent convulsions			100
Total = sum of all sections			

Best possible total Neurologic Deficit Score, 0; worst possible, 500.

rial cannula (Medtronic Inc, Minneapolis, Minn) was inserted in the ascending aorta, and a 36F venous single cannula (Terumo Co Ltd, Tokyo, Japan) was inserted in the right atrium. Extracorporeal circulation was performed by using a membrane oxygenator (Capiiox RX-Baby RX; 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 400 mL of lactated Ringer solution, 50 mL of 20% human albumin, 20 mL of sodium bicarbonate, 100 mL of mannitol, and 2500 IU of heparin. Cardiopulmonary bypass (CPB) was established at a flow rate of $100 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and the flow was 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. Animals were cooled to an esophageal temperature of 18°C by using a heat exchanger. The pH was maintained at 7.40 by means of pH-stat principles, with an arterial PCO_2 of 35 to 40 mm Hg, corrected for body temperature. CPB with perfusion cooling was performed for 45 minutes and was maintained for 10 minutes after reaching 18°C, before initiation of 120 minutes of deep hypothermic circulatory arrest (DHCA). The core and viscera temperatures were kept at 18°C by using ice packs and a cool room temperature. Cardiac arrest was induced and maintained by continuous infusion of cold cardioplegic solution after crossclamping of the ascending aorta.

After DHCA, CPB was restarted. Cardioversion was performed, if necessary, to resume the sinus rhythm at approximately

TABLE 2. Physiologic variables

Variable	Control	Erythropoietin	P value
Age (mo)	14.8 ± 2.0	15.8 ± 1.4	.83
Body weight (kg)	11.0 ± 0.3	11.9 ± 0.5	.30
Sex (male/female ratio)	3:2	3:2	.999
MAP (mm Hg)			
Preop	106.0 ± 1.0	102.4 ± 7.8	1.00
On CPB	56.0 ± 3.0	59.6 ± 5.4	.75
Post 3h	78.6 ± 7.6	65.8 ± 2.8	.14
Post 6h	82.2 ± 9.7	76.2 ± 4.2	.40
Post 12h	77.2 ± 9.6	70.0 ± 6.5	.75
CSF pressure (cm CSF)			
Preop	11.4 ± 0.6	12.4 ± 0.7	.38
On CPB	11.2 ± 1.2	11.8 ± 0.9	.92
Post 3h	29.8 ± 6.4	18.4 ± 3.6	.21
Post 6h	34.0 ± 16.8	17.4 ± 3.1	.075
Post 12h	43.0 ± 10.3	19.8 ± 3.3	.045*
Pao ₂ (mm Hg)			
Preop	495.1 ± 11.8	532.0 ± 25.5	.30
On CPB	528.7 ± 52.6	447.4 ± 55.5	.21
Post 3h	685.5 ± 30.6	628.4 ± 93.7	.83
Post 6h	530.4 ± 99.2	426.6 ± 134.3	1.00
Post 12h	388.2 ± 105.6	224.5 ± 112.3	.30
Paco ₂			
Preop	27.5 ± 4.4	30.9 ± 5.5	.53
On CPB	43.0 ± 4.2	41.4 ± 2.6	1.00
Post 3h	38.1 ± 4.2	28.0 ± 4.0	.10
Post 6h	35.6 ± 3.6	33.0 ± 3.8	.40
Post 12h	40.2 ± 3.3	44.5 ± 5.3	.53
Arterial pH			
Preop	7.44 ± 0.06	7.43 ± 0.05	1.00
On CPB	7.25 ± 0.02	7.29 ± 0.04	.67
Post 3h	7.29 ± 0.06	7.35 ± 0.05	.40
Post 6h	7.30 ± 0.04	7.34 ± 0.05	.46
Post 12h	7.23 ± 0.02	7.20 ± 0.03	.40
Svo ₂ (%)			
Preop	81.3 ± 5.1	81.5 ± 3.9	1.00
On CPB	99.5 ± 0.2	99.3 ± 0.6	.91
Post 3h	84.3 ± 6.0	83.7 ± 2.6	.40
Post 6h	68.2 ± 5.6	67.8 ± 8.1	1.00
Post 12h	40.8 ± 4.0	48.3 ± 8.7	.67
Hemoglobin (g/dL)			
Preop	12.5 ± 0.7	13.1 ± 1.1	.53
On CPB	5.4 ± 0.3	5.8 ± 0.3	.20
Post 3h	12.1 ± 1.0	12.5 ± 0.8	.68
Post 6h	11.3 ± 1.5	11.1 ± 1.7	1.00
Post 12h	10.3 ± 1.6	9.8 ± 1.8	1.00
Temp—esophagus (°C)			
Preop	34.9 ± 0.5	34.8 ± 0.5	.92
CA	17.3 ± 0.4	17.8 ± 0.2	.40
Post 3h	32.8 ± 0.5	32.4 ± 0.4	.67
Post 6h	33.7 ± 0.6	33.6 ± 0.4	.75
Post 12h	34.2 ± 1.1	34.3 ± 0.6	.92

TABLE 2. Continued

Variable	Control	Erythropoietin	P value
Temp—rectum (°C)			
Preop	34.5 ± 0.3	35.0 ± 0.6	.3
CA	18.0 ± 0.4	18.4 ± 0.4	.60
Post 3h	32.7 ± 0.3	32.8 ± 0.3	.83
Post 6h	33.9 ± 0.6	33.7 ± 0.4	.75
Post 12h	34.6 ± 0.4	34.2 ± 0.5	.67

Data are mean ± SEM. MAP, Mean arterial pressure; CSF, cerebrospinal fluid; Pao₂, arterial partial pressure of oxygen; Paco₂, arterial partial pressure of carbon dioxide; Svo₂, mixed venous saturation of oxygen; Temp, temperature; Preop, preoperative; on CPB, on cardiopulmonary bypass; Post 3h, 3 hours after operation; Post 6h, 6 hours after operation; Post 12h, 12 hours after operation; CA, circulatory arrest. *P < .05.

32°C, and mechanical ventilation was restarted. All animals were then slowly rewarmed to 37°C by using a heat exchanger and an infrared heater over 45 minutes and were slowly weaned from the CPB. After hemodynamic measurements, protamine was administered, and the CPB was removed. Autologous blood (removed before the operation) was then transfused. Finally, all wounds were closed; the animals were kept connected to the ventilator throughout the entire procedure. The ventilator mode was gradually stepped down to continuous positive airway pressure management to assess the animals' respiratory conditions. Anesthesia was kept at a minimum to enable the assessment of neurologic function until they awoke. At the moment of waking, they were anesthetized adequately again.

Neurologic Assessment

A postoperative neurologic assessment of each animal was performed by an independent observer who was a staff member of the Section of Animal Research and was unaware of the experimental groups. A modified Neurologic Deficit Score (NDS) in Dogs⁵⁻⁷ was used to evaluate the neurologic deficits. This measure evaluates 5 general neurologic components (central nerve function, respiration, motor and sensory function, level of consciousness, and behavior) on separate scales of 0 to 100. A total score of 500 indicates the worst possible neurologic damage, whereas a score of 0 is normal. All animals were closely evaluated for seizure activity. We modified this scoring by changing the "behavior" component to "abnormality" to enable the early postoperative condition of the animals to be evaluated (Table 1). A component of motor and sensory function that included the function of the hind limbs was used to assess the spinal cord.

Serum EPO Levels

Blood was sampled at several time points: before the operation, during CPB (rewarming), and 3, 6, and 12 hours after the operation. After centrifugation, the serum samples were stored at -80°C until analysis. Serum EPO concentrations were determined by using a commercially available radioimmunoassay kit (Riconbigen EPO kit; Mitsubishi Kagaku Iatron Inc, Tokyo, Japan).

CSF Evaluation

CSF was sampled at the same time points as the blood. The samples were centrifuged and stored at -80°C until analysis. The

Figure 1. Postoperative Neurologic Deficit Scores. * $P < .05$. The results for each of the 5 components were as follows (P value indicates EPO group vs control group): **A**, central nerve function, 15 ± 9.5 vs 90 ± 4.2 , $P = .011$; **B**, respiration condition, 0 ± 0 vs 50 ± 14 , $P = .0071$; **C**, motor and sensory function, 16 ± 7.5 vs 94 ± 6.0 , $P = .0092$; **D**, level of consciousness, 18 ± 7.3 vs 63 ± 10 , $P = .010$; **E**, abnormality, 10 ± 10 vs 80 ± 12 , $P = .015$.

tau protein concentration, a marker of neuronal damage, was then measured by using a commercially available enzyme-linked immunosorbent assay kit (Fino Scholar hTAU; Nipro, Osaka, Japan). The EPO concentration of the CSF was also measured by using the method described previously for the serum samples.

Histopathologic Examination

At 12 hours after the operation, cardiac arrest was induced under adequate anesthesia, and the brain and spinal cord were quickly harvested. The entire brain and spinal cord were fixed with 7% buffered formaldehyde solution. All coronal sections of the brain and all transverse sections of the spinal cord (5 mm thick) were examined for gross lesions, and the following sections were also investigated: frontal lobe; parietal lobe; thalamus; hippocampus CA1; cerebellum; cervical cord, C3-4; thoracic cord, T9-10; and lumbar cord, L3-4. These sections were embedded in paraffin, cut to a thickness of 10 μ m, stained with hematoxylin-eosin, and examined under a light microscope by a pathologist who was unaware of the experimental groups. During this early period after the onset of hypoxic ischemic injury, the minimum criteria for the diagnosis of ischemic neuronal damage included mild cytoplasmic eosinophilia, shrunken neurons with scalloping of the margins, and nuclear changes consisting of coarse nuclear chromatin or pyknosis.⁸⁻¹¹ A modified Histopathologic Damage Score (HDS)⁵ was used to evaluate the histopathologic damage. The score was defined as follows: no damaged neurons (0), minimal (2), mild (4), moderate (6), and severe (8).

Detection of Apoptosis

The extent of apoptosis in the brain (hippocampus CA1 and cerebellum) and spinal cord (thoracic [T9-10] and lumbar [L3-4]) tissue specimens was further examined by using a deoxyuridine-5'-triphosphate biotin nick end labeling (TUNEL) assay and the DeadEnd Colorimetric TUNEL System (Promega Corporation,

(A) EPO Levels in Serum

(B) EPO Levels in CSF

Figure 2. A, Erythropoietin (EPO) levels in serum. B, EPO levels in CSF. *preOp*, before surgery; *on CPB*, on cardiopulmonary bypass; *post 3h*, 3 hours after operation; *post 6h*, 6 hours after operation; *post 12h*, 12 hours after operation. * $P < .05$. The serum EPO levels (U/L) were significantly higher in the EPO group than in the control group during CPB ($15,390 \pm 3931$ vs 5.8 ± 0.5 ; $P = .011$) and at 3 hours ($13,077 \pm 3930$ vs 10.9 ± 2.1 ; $P = .012$), 6 hours (9506 ± 3482 vs 15.9 ± 4.0 ; $P = .012$), and 12 hours (5496 ± 2360 vs 13.3 ± 4.8 ; $P = .012$) after the operation. The EPO levels (U/L) in the CSF were also significantly higher in the EPO group than in the control group during CPB (176 ± 21 vs 5.3 ± 0.3 ; $P = .009$) and at 3 hours (3660 ± 1637 vs 6.8 ± 0.4 ; $P = .011$), 6 hours (1830 ± 1178 vs 8.1 ± 1.2 ; $P = .012$), and 12 hours (1865 ± 810 vs 8.9 ± 1.6 ; $P = .012$) after the operation.

Madison, Wis). Paraffin-embedded specimens were sectioned at 3 to 4 μ m for use in the TUNEL assay. Positive controls (specimens treated with deoxyribonuclease I) and negative controls (omission of recombinant terminal deoxynucleotidyltransferase) were also included. By using this procedure, apoptotic nuclei were stained dark brown with diaminobenzidine (TUNEL positive), and normal nuclei were counterstained blue-violet with hematoxylin (TUNEL negative). Two operators who were unaware of the experimental groups then counted the number of TUNEL-positive neurons in 3 separate high-power fields (magnification, 200 $\times$). The number of apoptotic neurons was scored on the basis of the percentage of the total number of neurons counted.^{1,2} The apoptotic score was defined as follows: no TUNEL-positive neurons (0), less than 10% (2), 10% to 30% (4), 31% to 50% (6), and greater than 50% (8).

Statistical Analysis

All data are presented as the mean $\pm$ SEM. Comparisons between the EPO and control groups were performed by using the nonparametric Wilcoxon test. The Spearman rank order correlation coefficient was used to correlate the 12-hour postoperative NDS and tau protein levels, as well as the NDS and HDS. All statistics were

Figure 3. Tau protein levels in CSF. *preOp*, Preoperative; *post 6h*, 6 hours after operation; *post 12h*, 12 hours after operation. * $P < .05$. The tau protein levels (pg/mL) were significantly lower in the EPO group than in the control group at both 6 hours (20 ± 12 vs 144 ± 54 ; $P = .036$) and 12 hours (64 ± 35 vs 478 ± 103 ; $P = .01$) after the operation.

computed by using 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 between groups. During the postoperative period, the CSF pressure was significantly lower in the EPO group at 12 hours after the operation ($P = .045$). No other parameters were significantly different between groups (Table 2). During this experimental period, no significant differences in the preoperative, intraoperative, and postoperative hemoglobin concentrations were observed between groups.

Neurologic Assessment

The final total NDS at 12 hours after the operation was significantly lower in the EPO group (59 ± 31) than in the control group (376 ± 30 ; $P = .012$; Figure 1). Hind-limb movement in the EPO group was significantly better than in the control group ($P = .015$): 2 dogs (40%) had weak hind-limb movement, and 3 dogs (60%) had normal hind-limb movement in the EPO group, whereas 1 dog (20%) had weak hind-limb movement and 4 dogs (80%) had no hind-limb movement in the control group.

Serum EPO Levels

The serum EPO concentrations before the administration of EPO or saline were similar in the EPO and control groups (11.8 ± 2.1 U/L vs 14.7 ± 2.4 U/L; $P = .68$). After the systemic administration of EPO or saline, the serum EPO levels were significantly higher in the EPO group than in the control group (Figure 2, A).

(A) Histopathologic Damage Score

(B) Apoptotic Score

Figure 4. A, Histopathologic Damage Score (HDS). B, Apoptotic score. FL, Frontal lobe; PL, parietal lobe; THA, thalamus; HIPCA1, hippocampus CA1 area; CER, cerebellum; C, cervical cord; Th, thoracic cord; L, lumbar cord; TOTAL, total HDS. * $P < .05$. The regional HDS values for the 8 anatomic areas were as follows: frontal lobe (1.6 ± 0.2 vs 3.2 ± 0.5 ; $P = .041$), parietal lobe (1.2 ± 0.4 vs 4.4 ± 0.2 ; $P = .01$), thalamus (1.6 ± 0.2 vs 4.8 ± 0.5 ; $P = .0097$), hippocampus CA1 (1.4 ± 0.2 vs 6.0 ± 0.3 ; $P = .0099$), cerebellum (3.6 ± 0.7 vs 7.0 ± 0.4 ; $P = .019$), cervical cord (4.0 ± 0.6 vs 6.4 ± 0.7 ; $P = .064$), thoracic cord (3.2 ± 0.8 vs 6.0 ± 0.6 ; $P = .049$), and lumbar cord (4.8 ± 0.5 vs 6.8 ± 0.5 ; $P = .041$). The apoptotic scores in the hippocampus CA1 and cerebellum were significantly lower in the EPO group than in the control group (2.8 ± 0.5 vs 6.0 ± 0.3 [$P = .029$] and 2.8 ± 0.5 vs 6.0 ± 0.6 [$P = .017$], respectively).

EPO Levels in the CSF

The EPO concentration in the CSF before the administration of EPO or saline was similar in the EPO and control groups (8.2 ± 0.7 U/L vs 7.3 ± 0.6 U/L; $P = .75$). After the systemic administration of EPO or saline, the EPO levels in the CSF were significantly higher in the EPO group than in the control group (Figure 2, B).

Tau Protein Levels in the CSF

The tau protein concentrations in the CSF were measured before the operation and 6 and 12 hours after the operation. No significant differences in the tau protein concentrations before the operation were seen between the EPO and control groups (44.2 ± 13.6 pg/mL vs 39.4 ± 6.0 pg/mL; $P = .60$). The tau protein levels were significantly lower in the EPO group than in the control group after the operation (Figure 3).

A significant correlation between the 12-hour postoperative total NDS and tau protein levels was seen (Spearman correlation coefficient [r] = 0.7622; P = .011).

Histopathologic Examination

No macroscopic gross lesions were observed in any of the brain or spinal cord specimens. The total HDS was significantly lower in the EPO group than in the control group (21 ± 2.6 vs 45 ± 2.1 ; P = .012). Regional HDS, except for the cervical cord, showed similar significant group differences (Figure 4, A). A significant correlation between the total NDS and the HDS was observed (Spearman correlation coefficient [r] = 0.7669; P = .0096). Samples from both groups are shown in Figure 5, A-D.

Detection of Apoptosis

The apoptotic scores in the hippocampus CA1 and cerebellum were significantly lower in the EPO group than in the control group. The differences in the apoptotic scores of the spinal cord specimens were not significantly different between the 2 groups. However, the lumbar cord score tended to be lower in the EPO group than in the control group (4.0 ± 0 vs 5.2 ± 0.5 ; P = .067; Figure 4, B). Samples from the control group stained positive (dark brown with diaminobenzidine) with numerous TUNEL-positive neurons scattered in the hippocampus and cerebellum compared with samples from the EPO group (Figure 5, E-H).

Discussion

EPO is known mainly for its ability to stimulate the production of red blood cells in bone marrow. EPO expression is increased in the kidneys whenever low levels of oxygen occur, a condition sensed by the transcription factor hypoxia-inducible factor 1. EPO is used to treat anemia in patients with chronic renal failure.

EPO is also produced in the brain after oxidative stress. Moreover, neurons express receptors that detect EPO.¹ Recent studies have shown that EPO protects the brain and spinal cord from ischemic injury by preventing apoptosis.^{3,12-14} In this study, a TUNEL assay showed that EPO can rescue neurons from apoptosis in selective vulnerability areas,^{10,11} such as the hippocampus CA1 (Sommer sector) and the Purkinje cells of the cerebellum. Neurons in the lumbar cord also tend to be rescued by EPO. Apoptosis, or programmed cell death, is a kind of delayed neuronal death that occurs 24 hours to 2 weeks after an insult.

This study revealed that EPO also significantly decreased acute ischemic neuronal cell changes (microvacuolization, pyknotic nuclei, dense staining triangular nuclei, and shrunken eosinophilic cytoplasm). These findings suggest that EPO may also prevent necrosis, because acute ischemic neuronal cell changes reflect early necrotic changes. We speculated that EPO may prevent brain edema caused by ischemic

Figure 5. (A) and (B) are sections of the hippocampus CA1 area (stain, hematoxylin and eosin; original magnification, 200 $\times$). (C) and (D) are sections of the cerebellum (stain, hematoxylin and eosin; original magnification, 200 $\times$). (A) and (C) are from the EPO group and show minimal evidence of cellular change. (B) and (D) are from the control group and show pyknotic nuclei, shrunken eosinophilic cytoplasm, and microvacuolization. (D) also shows a decrease in the number of Purkinje cells. TUNEL staining of sections of the hippocampus CA1 in the EPO group (E) and the control group (F) and of the cerebellum in the EPO group (G) and the control group (H) is shown (original magnification, 200 $\times$). (E) and (G) show minimal evidence of TUNEL-positive neurons, whereas (F) and (H) exhibit numerous scattered TUNEL-positive neurons (dark brown with diaminobenzidine).

neuronal cell change (microvacuolization), thus leading to a lower CSF pressure, because the CSF pressure has been previously shown to reflect the intracranial pressure.¹⁵ The tau proteins observed in our study were thought to be produced by ischemic damaged neurons. Brines and colleagues¹² concluded that EPO could rescue neurons from

death through not only the modulation of apoptosis, but also the modulation of necrosis or immune-mediated injury. In their study, EPO was thought to play an acute neuroprotective role in apoptosis modulation by activating gene expression. Siren and colleagues³ also conjectured that EPO might limit neuronal necrosis. Further experiments will be necessary to determine the mechanism by which EPO prevents necrosis.

Romsis and associates¹⁶ showed the potential neuroprotective benefits of EPO during hypothermic circulatory arrest but failed to show any significant benefit in terms of mortality or brain histopathology. The dose of EPO that they used (500 U/kg of body weight) might have been inadequate. The dose used in this study (5000 U/kg of body weight) has also been reported to have good neuroprotective effects in other articles.^{3,17} Our data confirmed that intravenously administered EPO (a genetic recombination form) was able to cross the blood-brain barrier during CPB and DHCA.

Although EPO's ability to protect the central nervous system may be clinically useful, its erythropoietic activity would be undesirable in patients with cardiovascular or cerebrovascular diseases. Recent studies have shown that nonerythropoietic variants of EPO (such as carbamylated EPO¹³ or asialo EPO¹⁴) may also provide tissue-protective effects.

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, the long-term survival of brain-damaged and laminectomized animals would be neither feasible nor humane. Therefore, we decided to limit our postoperative observations to a 12-hour period.

Conclusion

This study showed that EPO protects the central nervous system during prolonged hypothermic circulatory arrest, partly by preventing both necrosis and apoptosis. This result suggests that EPO may be useful for preventing neurologic injury after aortic surgery.

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