Conditioned blood reperfusion markedly enhances neurologic recovery after prolonged cerebral ischemia

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Objectives: To determine whether controlled reperfusion using conditioned leukodepleted blood can substantially limit cerebral reperfusion injury following prolonged ischemia.

Methods: Eighteen pigs (25-35 kg) underwent 90 minutes of hypothermic circulatory arrest (19°C) to produce brain ischemia. At the start of rewarming, 10 pigs received uncontrolled reperfusion with unmodified (normal) blood. The other 8 pigs underwent 10 minutes of controlled reperfusion by selectively perfusing both common carotid arteries with blood passed through a CoBRA filter. This filter conditions the blood by removing white blood cells, platelets, and attenuating complement. Two other pigs underwent cooling and rewarming only (controls) without ischemia. Neurologic assessment was done using neurologic deficit scoring (0 = normal, 500 = brain death), and jugular venous samples were obtained for biochemical analysis postreperfusion.

Results: There were no statistical differences in hemodynamics between groups. At 6 hours postanesthesia, all animals receiving normal blood were substantially neurologically impaired. At 24 hours, they all had abnormal positioning and all but 1 were unable to sit or stand (neurologic score 124 ± 19). In contrast, nonischemic controls and pigs receiving conditioned blood reperfusion showed only minor neurologic deficits at 6 hours, and at 24 hours all were considered normal (neurologic scores 0 and 6 ± 5 ; $P < .005$ vs uncontrolled reperfusion). Compared with pigs receiving normal blood reperfusion, oxygen free radical formation (conjugated dienes 1.70 ± 0.03 vs 1.60 ± 0.02 Abs 240 nm; $P < .05$ vs uncontrolled reperfusion), and endothelin-1 release (2.12 ± 0.09 vs 1.84 ± 0.06 pg/mL; $P < .05$ vs uncontrolled reperfusion) were also significantly lower in animals receiving conditioned blood.

Conclusions: Following prolonged cerebral ischemia, reperfusion injury is avoided by delivering conditioned blood, which is devoid of white cells, platelets, and membrane attack complex. These results suggest that this modality is clinically useful in situations where the brain is subjected to prolonged ischemia.

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Ischemia is a passive and time-dependent phenomenon caused by insufficient oxygen and nutrient supply, whereas reperfusion injury is an active process that results in functional, metabolic, and structural alterations with the reintroduction of blood.¹⁻⁵ It is clear that cells appearing undamaged at the end of ischemia may undergo severe injury following reperfusion with unmodified blood.^{1,2} The consequence is that of impaired organ function and increased morbidity and mortality if ischemia is prolonged. Nevertheless, studies in the heart, lung, kidney, and leg show reperfusion damage can be coun-

teracted by control of the composition of the reperfusate and conditions of reperfusion.^{1,2,4,6-11} Most recently, recovery of cerebral function was demonstrated following deep hypothermic circulatory arrest, where global reperfusion of the entire body by modifying the pump prime reperfusate favorably improved brain function.¹² Unfortunately, the extent of recovery was not evaluated beyond the acute setting, and specific contributions of different components of the reperfusate solution were not analyzed. Moreover, there was no regional control of cerebral reflow to focus upon selective resuscitation of the brain, the organ most sensitive to ischemia and reperfusion damage.

Reperfusion injury can be mediated by activated constituents of blood, including leukocytes (white blood cells [WBCs]), especially neutrophils, platelets, and complement, specifically the distal components of the complement cascade (C5b-9, membrane attack complex).^{3,11,13-23} Each element can serve as a target of pharmacological or physical interventions designed to reduce reperfusion injury, since attenuation of reperfusion injury was achieved in myocardial and pulmonary tissue by removal of activated WBCs, platelets, or membrane attack complex. However, the importance of these elements in brain reperfusion injury is uncertain.

The present study will determine the degree of involvement of these elements in the reperfusion injury of the brain, using a model of hypothermic circulatory arrest (HCA) to produce cerebral ischemia. This global model was used to produce cerebral ischemia because most nonprimates have extensive neurologic vascular collateral networks, which are not uniformly injured by unilateral or bilateral carotid ligation.^{12,24} Following this ischemic insult, either the brain was reperfused in an uncontrolled manner with normal (unmodified) blood or reperfusion was controlled by selectively perfusing the carotid arteries with blood that was first passed through a Conditioned Blood Reperfusion Application (CoBRA) filter (Pall B-1328 filter, Pall Corporation, East Hills, NY). This filter selectively removes virtually all WBCs and platelets, as well as attenuates various complement proteins including membrane attack complex.^{12,25} The combined effect on WBCs and platelet removal as well as the complement-modulating effect of this filter suggest that this multifactorial approach may provide an optimal way to attenuate reperfusion injury.

Our initial study end point aimed at the potential for functional neurological recovery (using a well-recognized conventional scoring system) after a 24-hour time frame, to allow dissipation of the contributory mental effects of extracorporeal circulation and anesthesia.^{12,26} Our secondary end point of biochemical and hemodynamic recovery was also evaluated but an intact functional neurologic system is the landmark of avoidance of brain injury.

Material and Methods

Twenty pigs (2-3 months old, 25-35 kg) pigs were premedicated with 15 mg/kg ketamine and 0.5 mg/kg diazepam intramuscularly. They were then anesthetized with inhaled isoflurane and fentanyl (5 ug/kg), and the lungs were ventilated using a volume ventilator. All animals received humane care in compliance with the Principles of Laboratory Animal Care published by the National Institutes of Health (publication no. 96-03, revised 1996).

Using sterile techniques, the thoracic artery and vein were cannulated to monitor arterial pressure and for use in blood gas determinations and intravenous infusions. After systemic heparinization (3 mg/kg) a 10- to 12-Fr thin-walled cannula was inserted into the femoral artery, a 8- to 20-Fr short venous cannula placed into the external jugular vein, and a Swan-Ganz catheter placed into the pulmonary artery via the internal jugular vein. Arterial blood gases, electrolytes, and hemoglobin (Blood Gas System 288, Ciba Corning, Midfield, Mass) were measured every 15 to 30 minutes to ensure optimal levels. A heating/cooling blanket was used to maintain the desired core temperature. The cardiopulmonary bypass circuit was heparinized, primed with packed red cells from donor pigs, and made normocalcemic. Hematocrit value was adjusted to 25% to 35% with plasmalyte solution. An Affinity NT 541 membrane oxygenator (Medtronic, Inc, Minneapolis, Minn) was used and the systemic flow rate adjusted to approximately 100 to 125 mg/kg/min at full flow to maintain continuously monitored aortic root pressures of 50 to 70 mm Hg. To facilitate venous return, active suction by a centrifugal pump (Biopump BPX-80, Medtronic, Inc) was maintained during bypass.

Experimental Protocol

All animals were placed on cardiopulmonary bypass using 100% FIO₂, cooled to 19°C using an alpha-stat strategy, and placed into 3 experimental groups. In 2 pigs, cardiopulmonary bypass (CPB) was continued without ischemia (controls), and the other 18 underwent 90 minutes of HCA. Following HCA, bypass was restarted at a pressure of 20 to 30 mm Hg for 10 minutes (in all pigs) and then slowly increased to 50 to 70 mm Hg. All pigs were rewarmed to 36°C to 37°C. The heart was defibrillated as needed at 25°C and all pigs were started on 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of dopamine when core temperature reached 35°C. Dopamine was then either increased or weaned to keep systolic arterial pressure above 70 mm Hg and was discontinued only when arterial pressure was maintained greater than 70 mm Hg for 5 minutes. Hemodynamic measurements were obtained before (baseline) and 30 minutes post-CPB. Biochemical measurements were made from samples taken from the internal jugular vein 5 minutes after initiating CPB (baseline), immediately prior to hypothermic (19°C) circulatory arrest, during rewarming when the temperature reached 36°C to 37°C, and at 4 and 24 hours post-CPB. Following the hemodynamic measurements, protamine was given and all cannulas removed. Bleeding was controlled, the vessels repaired, and all wounds closed. The animals were then allowed to regain consciousness and were extubated; they underwent neurologic assessment approximately 6 and 24 hours later. Fentanyl (0.2-0.3 ug/kg) was given as needed for postoperative pain but was held for at least

2 hours prior to the neurologic evaluation. The animals were then killed.

Experimental Groups

Controls To distinguish the effects of extracorporeal circulation alone without ischemia, 2 pigs underwent cooling to 19°C and immediate rewarming to 37°C.

Hypothermic circulatory arrest After cooling to 19°C, the other 18 pigs underwent 90 minutes of hypothermic circulatory arrest and were reperfused using either the unmodified (normal) blood prime (uncontrolled reperfusion) or selective carotid reperfusion (controlled reperfusion) with conditioned blood.

Unmodified blood (uncontrolled) reperfusion Following HCA, CPB was reinstituted in 10 pigs (pressure 20-30 mm Hg) without altering the pump blood bypass prime. This simulates the conventional clinical method to reperfuse the brain following ischemia.

Conditioned blood (controlled) reperfusion In 8 pigs, the common carotid arteries were exposed during circulatory arrest. Each received a carotid artery graft, harvested from blood donor pigs, into each carotid artery. A "Y"-shaped cannula was inserted into the tip of each graft to selectively reperfuse both vessels at the initiation of CPB, which started at the beginning of rewarming (initiation of CPB). Each pig underwent 10 minutes of selective (regional) common carotid perfusion with white cell and platelet-free, complement attenuated blood by passing the blood prime through 6 parallel CoBRA filters (Pall B-1328 filter, Pall Corporation). A pressure-tip needle was inserted into the carotid graft to monitor perfusion pressure, which was kept at 30 ± 4 mm Hg, ensuring at least 200 mL/min reflow. During regional carotid perfusion, systemic blood (CPB) pressure was maintained at 20 mm Hg to minimize unfiltered collateral brain blood flow via vertebral vessels. At the end of controlled reperfusion, the grafts were closed by clipping them above the anastomosis to restore carotid flow through the pig's native carotid vessel.

Physiologic and Biochemical Determinations

Hemodynamic measurements Hemodynamic measurements were made before and 30 minutes after discontinuing CPB. Cardiac output was determined by duplicate injections of 4°C cold saline solution into the Swan-Ganz catheter. Cardiac output and pulmonary vascular resistance were calculated as previously described.^{7,27} Dopamine requirements are expressed as the total time each animal received inotropic support.

Conjugated dienes Conjugated dienes, a marker of oxidant-mediated lipid peroxidation, were determined as previously described and expressed as the absorbency (A) at a wave length of 240 nm/0.5 mL plasma.^{12,27}

Creatinine kinase and serum transaminase Cellular injury was determined by measuring creatinine kinase (CK) and serum transaminase (AST) activity by the ultraviolet spectrophotometric method (Sigma Chemical Co, St Louis, Mo) and expressed as U/mL plasma.

Endothelin-1 Vascular injury was determined by measuring endothelin levels by an Enzyme Immunometric Assay (ACE EIA Kit, Cayman Chemical Co, Ann Arbor, Mich) and expressed as pg/mL plasma.

TABLE 1. Neurologic deficit scoring

	Side*	Normal	Weak	Absent
Central nerve function				
Pupil size	R, L	0	2	5
Light reflex	R, L	0	2	5
Eye position	R, L	0	2	5
Lid reflex	R, L	0	2	5
Corneal reflex	R, L	0	2	5
Ciliospinal reflex	R, L	0	2	5
Occulocephalic reflex		0	5	10
Auditory reflex		0	5	10
Gag reflex		0	5	10
Carinal reflex		0	5	10
Respiration				
Normal				0
Hyperventilation				25
Abnormal				50
Apnea				100
Motor and sensory function				
Stretch reflex		0	10	25
Motor response to pain		0	10	25
Positioning		0	10	25
Muscle tonus		0	10	25
Level of consciousness				
Normal				0
Cloudy				30
Delirium				45
Stupor				60
Coma				100
Behavior				
Drinking		0	—	15
Chewing		0	—	15
Sitting		0	—	15
Standing		0	—	15
Walking		0	20	40

R, Right; L, left. Total = sum of all sections (0 = normal, 500 = brain death).

*Score each side separately.

Neurologic injury Neurologic assessment was performed in all animals 6 hours after wounds were closed and after the animal was extubated (approximately 7 hours post-CPB). The last 5 animals in each group also underwent detailed neurologic evaluation at 24 hours. An adaption of neurologic deficit scoring described elsewhere was used, and a summary of the variables assessed are listed in Table 1.^{12,26} In neurologic deficit scoring, 5 general components of the neurologic examination are evaluated and a score of 100 is assigned to each category. A total score of 500 indicates brain death and a score of 0 is normal. Neurologic deficit scoring was always agreed upon by 2 members of the laboratory team.

Statistics

All data are expressed as mean $\pm$ SE. Statistical analysis of data within and between groups was performed using multiple analysis of variance followed by application of the Student *t* test with Tukey-Kramer correction for multiplicity. Data at 24 hours was analyzed using nonparametric Wilcoxon test. Changes within and between groups were considered statistically significant when the *P* value was less than .05.

TABLE 2. Hemodynamic data

	MAP (mm Hg)	Heart rate	MPAP (mm Hg)	LAP (mm Hg)	Cardiac output (L/min)	LVSWI	SVR	PVR	Dopamine (min)
Baseline									
No HCA (controls)	67	107	15	9	3.9	28	1200	143	
Normal reperfusion	66 ± 4	94 ± 4	13 ± 1	6 ± 1	4.2 ± 0.6	33 ± 6	1327 ± 174	153 ± 28	
Conditioned reperfusion	57 ± 3	93 ± 3	12 ± 1	6 ± 1	3.7 ± 0.5	30 ± 5	1214 ± 130	149 ± 34	
After CPB									
No HCA (controls)	53	150	18	7	3.2	12	1015	130	0
Normal reperfusion	68 ± 7	144 ± 15	20 ± 2	5 ± 1	4.2 ± 0.5	25 ± 4	1195 ± 176	343 ± 73	134 ± 22
Conditioned reperfusion	62 ± 5	134 ± 11	25 ± 3	7 ± 1	3.8 ± 0.6	26 ± 4	1318 ± 202	492 ± 123	145 ± 20

MAP, Mean arterial pressure; MPAP, mean pulmonary artery pressure; LAP, left atrial pressure; LVSWI, left ventricular stroke work index; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; HCA, hypothermic circulatory arrest; CPB, cardiopulmonary bypass.

TABLE 3. Neurologic evaluation 24 hours postanesthesia

	Normal blood reperfusion	Conditioned blood reperfusion
Level of consciousness		
Normal	2 (40%)	5 (100%)
Cloudy	3 (60%)	0
Motor/sensory function		
Abnormal positioning	5 (100%)	0
Behavior		
Drinking	0	5 (100%)
Chewing	5 (100%)	5 (100%)
Sitting	2 (40%)	5 (100%)
Standing	1 (20%)	5 (100%)
Walking	0	5 (100%)

Data expressed as absolute number and percentage of animals of each group.

Results

Results are shown in Tables 2 and 3 and Figures 1 and 2. There were no statistical differences ($P > .2$) between normal and conditioned blood reperfusion groups for cooling (41 ± 8 and 38 ± 10 minutes) or rewarming time (82 ± 17 and 87 ± 19 minutes).

Hemodynamic and Pulmonary

All pigs remained in stable condition through out the experimental procedure. Hemodynamic parameters were similar in both groups before and after CPB (Table 2). Dopamine was consistently required (range 58-240 minutes post-CPB) with no differences ($P > .2$) between ischemic groups. Oxygen administration was required in most animals for 2 hours after extubation, with no differences between groups.

Biochemical

No difference between groups ($P > .2$) was noted for control (baseline) values of conjugated dienes (0.90 ± 0.03 Abs 240 nm), endothelin-1 (0.94 ± 0.04 pg/mL), creatinine kinase (383 ± 23 u/L), or AST (30 ± 3 u/L). None of the

parameters measured were modified by cooling to 19°C. Conjugated dienes and endothelin-1 levels remained within baseline levels in pigs not undergoing ischemia (controls), whereas a twofold rise was observed in animals undergoing circulatory arrest. However, both conjugated dienes and endothelin levels were appreciably higher in pigs receiving unmodified blood reperfusion (Figure 1, A and B). Parameters of cellular injury also rose after ischemia. CK levels rose in all groups, probably as a result of limb ischemia from femoral cannulation (Figure 1, C). It was further increased in animals undergoing circulatory arrest. Nevertheless, CK was lower in pigs receiving conditioned blood compared with those receiving unmodified blood. Postreperfusion, the AST levels rose to 265 ± 12 U/mL and 259 ± 11 U/mL in animals receiving unmodified and conditioned blood, respectively, compared with 123 U/mL in the control group, with no statistical differences ($P > .2$) between ischemic (HCA) groups. These levels declined to 181 ± 6 and 167 ± 6 by 24 hours.

Neurologic Function

Postbypass neurologic function is depicted in Table 3 and Figure 2. Extubation time after weaning from anesthesia was similar in all groups. Pigs undergoing cooling and immediate rewarming with no ischemia (controls) developed no significant neurologic deficits at 6 hours postanesthesia. In them, neurological recovery was almost normal, with only unbalanced walking noted, and by 24 hours it was considered normal (neurologic score 0).

In contrast, animals subjected to 90 minutes of hypothermic arrest followed by reperfusion with normal blood (uncontrolled reperfusion) presented with major neurological deficits at 6 hours, and these defects were still present at 24 hours (neurologic score 124 ± 19). No pig was able to walk or drink, and only 1 pig was able to stand.

These adverse effects of uncontrolled reperfusion were completely reversed by use of a conditioned reperfusate. Pigs receiving controlled reperfusion of the carotid arteries

Figure 1. Oxidant-mediated lipid peroxidation measured as conjugated dienes (A), endothelin-1 (B), and creatinine kinase (C) plasma levels in internal jugular vein at different stages of the experiment, depending on the type of carotid reperfusion. * $P < .05$ between ischemic groups. HCA, Hypothermic circulatory arrest.

with conditioned blood presented recovery of neurologic findings that were similar to pigs undergoing no brain ischemia (controls). They presented only mild neurologic impairment at 6 hours and the examination was essentially normal at 24 hours, with each pig able to walk and feed himself (neurologic score 6 ± 5).

Discussion

This study shows that reperfusion injury is a major factor of neurologic damage after an ischemic insult and that it can be reduced by controlling the initial period of reperfusion with conditioned blood devoid of leukocytes, platelets, and complement (membrane attack complex). The improved, near-normal neurologic function was also associated with reduced formation of oxygen free radicals and vascular injury. These findings imply that WBCs, platelets, and complement play an important role in triggering neurologic damage because pigs receiving normal blood reperfusion demonstrated major neurologic deficits. Although we did not extend this observation time beyond 24 hours, previous data using similar circulatory arrest times and longer follow-up did not demonstrate reversibility of this brain injury.^{26,28,29}

There is, of course, a limitation of our observations in this pilot study as we did not examine the brain histologically or evaluate specific biochemical markers of injury (ie, S-100 protein, neuron-specific enolase). Neurologic deficit scoring, however, directly correlates with pathologic examination and the release of biochemical markers of injury and more importantly is the hallmark of brain recovery.^{26,28-30}

Figure 2. Neurologic injury 6 and 24 hours after discontinuing cardiopulmonary bypass assessed by neurologic deficit scoring. A score of 0 equals normal and 500 equals brain dead. Scoring system is shown in Table 1. * $P = .02$ at 6 hours and $P < .005$ at 24 hours.

Our findings show that pigs who received conditioned blood were neurologically completely normal, implying no major pathologic damage.

Moreover, a significant reduction in neurologic damage after circulatory arrest has been demonstrated after WBC filtration alone in chronic animals observed for several days.²⁸ However, these investigators used a relatively inefficient WBC filter. In contrast, we suspect that using a CoBRA filter, which is not only more effective in removing WBCs but also removes platelets and attenuates complement, should vastly improve the results, as others have shown that more effective WBC filters dramatically reduce damage.^{2,4,7,10,12,17,25,31}

WBCs are involved mainly in the maintenance of the immune system but under the altered conditions of normal blood reperfusion after ischemia may cause damage to myocardial, pulmonary, or vascular tissue.^{4,7,17,31} Leukocyte depletion is a readily available method that allows the surgeon to safely minimize the harmful effects of neutrophils without risking side effects of pharmacologic interventions aimed at altering leukocyte function or preventing the free radical injury through the use of exogenous oxygen radical scavengers. WBCs have a variety of deleterious effects.^{4,7,12,17,25,31,32} Under conditions of hypoxemia or ischemia, vascular endothelium expresses sites that bind neutrophils on reperfusion. Once bound, the neutrophil may be activated by several different mechanisms. The bound activated neutrophil is then involved in pathways that contribute to cellular injury, as well as releasing substances that are chemotactic for other neutrophils and macrophages, resulting in an amplification of the inflammatory response.

Although WBCs can injure tissue by several mechanisms, numerous studies have demonstrated the production of oxygen radicals is probably the major factor responsible for cellular damage.^{4,7,10,17,31,32} This explains the reduction in oxygen free radical formation (lower conjugated dienes) in pigs receiving leukodepletion, as well as the reduction in vascular damage (decreased endothelin-1) because activated WBCs cannot cause damage to the vascular endothelium if they are removed.

Platelets may act synergistically with WBCs to promote reperfusion injury.^{12,17,21,23,25,33} Activated platelets and WBCs form aggregates that may restrict blood flow in vascular beds. In addition, platelet factor 3 as well as platelet-derived microparticles stimulate neutrophils. Platelets can also release harmful substances such as thromboxane, which can lead to vasoconstriction and other damaging effects that can further promote cellular damage. These considerations make platelet reduction a desirable property of blood conditioning.

The third component in conditioned blood is complement. Plasma is operative in development of reperfusion injury and can augment the leukocyte-mediated components of reperfusion injury.^{18-20,22,25} Complement activation occurs during routine CPB and further activates WBCs and platelets, increasing cellular injury in the setting of reperfusion. Several components of the complement cascade are anaphalotoxins but the distal component (C5a-9, membrane attack complex) is the most damaging, as it can injure cellular membranes directly and increases cell membrane permeability.¹⁸⁻²⁰ Moreover, membrane attack complex has an important function in mediating the recruitment of neutrophils to the reperfused cell through local induction of interleukin-8 (IL-8). Membrane attack complex deposition may increase the concentration of the endothelial cell-de-

rived neutrophil chemotactant, IL-8, and blocking this may attenuate WBC extravasation into the interstitial space.

Conditioned blood reperfusion of the carotid arteries was accomplished using a CoBRA filter. This filter reduces leukocytes and platelets by 99.99% and attenuates or removes complement proteins, especially membrane attack complex.^{12,25} The combined WBC/platelet reduction and complement attenuating blood conditioning effect provides an ideal technological approach to positively impact on reperfusion injury. However, the CoBRA filter can only accommodate flows up to 100 to 150 cc/min and becomes fully saturated after 500 to 750 cc of blood. Therefore, we pumped the blood through 6 filters in a parallel system before being administered to both common carotid arteries through a "Y" cannula and arterial grafts. This system allows up to 600 mL/min flow with a maximum filtration capacity of 3 L.

Only the blood components of the reperfusate process were altered in this study but the benefits of controlled reperfusion are consistent with our prior studies, where the reperfusion damage caused by restoring normal blood supply to the kidney, heart, lung, extremity, and whole body was offset by controlling both blood components and other metabolic and mechanical factors during the initial period of reperfusion.^{1,2,4,6-12} Nevertheless, controlled reperfusion is not just removing the various blood components but changing the composition of the reperfusate and the conditions of reperfusion.^{1,2,4} Modifying constituents such as oxygen, glucose, osmolarity, pH, calcium, and magnesium would probably also enhance neurologic recovery but was not tested in these studies so that we could define the effect of the blood components without other confounding variables.

A lower initial reperfusion pressure (20-30 mm Hg) reduces cellular damage in the heart, lung, and brain.^{2,4,11,34} This lower pressure was used in both groups of pigs undergoing ischemia, both to keep blood filtration as the only variable and to reduce collateral flow via vertebral vessels. We did not study a higher perfusion pressure, which is conventionally used when an occluded artery is acutely reopened, so that the effects of limiting this variable were not tested. Nevertheless, we strongly believe, based on our prior experience, that modifying the composition of the reperfusate as well as the conditions of reperfusion (ie, pressure) are vital adjuncts in limiting reperfusion damage and should always be use in concert with CoBRA filtration.

A model of 90 minutes of circulatory arrest was used to produce brain ischemia, as most nonprimates have extensive neurologic vascular collaterals.²⁴ Ligating 1 or both carotid arteries often results in no significant brain ischemia so that the initiating insult is not consistent. Therefore, circulatory arrest became a reliable way to produce a consistent and uniform cerebral insult in the pig. This model has also been used by other investigators and results in a rela-

tively homogeneous neurologic insult.^{26,28,29,35} We suspect this insult mimics acute regional cerebral ischemia (ie, arterial occlusion) in primates, and we hope the findings can be applicable to any form of brain ischemia. The complete neurologic recovery is a new and previously unreported finding and sets the stage for further studies of longer intervals of brain ischemia, where this aspect of controlled reperfusion can both be extended and, more importantly, possible integration with other methods of reducing reperfusion damage.

Plasma levels of conjugated dienes and endothelin-1 were significantly lower in the conditioned blood group, suggesting that oxygen radical injury and vascular injury were better controlled. Nevertheless, the biochemical results may have been misleading and almost certainly underestimated the improvement with CoBRA filtration. Blood was taken from the jugular vein only after rewarming to 37°C. By this time, a generalized reperfusion injury would have taken place in every organ, releasing oxygen free radicals (conjugated dienes), endothelin (vascular damage), CK, and AST into the systemic pump blood. Therefore, the samples from both ischemic groups presented higher levels than control nonischemic animals, as elevations in jugular venous samples reflected the higher circulating levels produced by other organs. Systemic perfusion was initiated at the same time as selective carotid perfusion, and so it is possible that some unmodified (unfiltered) blood perfused portions of the brain, increasing the neurologic injury. Jugular venous samples may also be contaminated by drainage from nonneurologic tissue. Sampling immediately after reperfusion or obtaining simultaneous cerebral arterial and venous samples would have been a better way of specifically quantifying brain production but this was not done. Nevertheless, the data support a reduction in brain injury, as any differences in biochemical levels must reflect a limitation of cerebral injury because conditioned blood was only delivered to the brain.

In conclusion, this study confirms that reperfusion injury is a major cause of neurological cell damage after an ischemic insult and demonstrates that reperfusion injury can be significantly limited by controlling the initial period of reperfusion using conditioned blood devoid of WBCs, platelets, and complement. Incorporating this modality during direct cerebral reperfusion may allow surgeons to reduce the damage following cerebral ischemia, potentially leading to a significant reduction in morbidity and mortality and improved patient outcome.

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