Deep hypothermic circulatory arrest and global reperfusion injury: Avoidance by making a pump prime reperfusate—A new concept

Bradley S. Allen, MD
Jeffrey S. Veluz, MD
Gerald D. Buckberg, MD
Ernesto Aeberhard, MD, PhD
Louis J. Ignarro, MD

See related editorial on page 460.

From the Division of Cardiovascular Surgery, University of California at Los Angeles Medical Center, Los Angeles, Calif, and The Heart Institute for Children, Hope Children's Hospital, Oak Lawn, Ill.

Read at the Eighty-first Annual Meeting of The American Association for Thoracic Surgery, San Diego, Calif, May 6-9, 2001.

Received for publication May 16, 2001; revisions requested July 18, 2001; revisions received Sept 19, 2001; accepted for publication Oct 2, 2001.

Address for reprints: Bradley S. Allen, MD, The Heart Institute for Children, Hope Children's Hospital, 4440 West 95th St, Oak Lawn, IL 60453 (E-mail: bradallen@thic.com).

J Thorac Cardiovasc Surg 2003;125:625-32

Copyright © 2003 by The American Association for Thoracic Surgery

0022-5223/2003 \$30.00+0

doi:10.1067/mtc.2003.96

Objective: We sought to determine whether damage after deep hypothermic circulatory arrest can be diminished by changing pump prime components when reinitiating cardiopulmonary bypass.

Methods: Fifteen piglets (2-3 months old) were cooled to 19°C by using the alpha-stat pH strategy. Five were cooled and rewarmed without ischemia (control animals), and the other 10 piglets underwent 90 minutes of deep hypothermic circulatory arrest. Of these, 5 were rewarmed and reperfused without altering the cardiopulmonary bypass circuit blood prime. In the other 5 animals, the bypass blood prime was modified (leukocyte depleted, hypocalcemic, hypermagnesemic, pH-stat, normoxic, mannitol, and an Na⁺/H⁺ exchange inhibitor) during circulatory arrest before starting warm reperfusion. Oxidant injury was assessed on the basis of conjugated dienes, vascular changes on the basis of endothelin levels, myocardial function on the basis of cardiac output and dopamine need, lung injury on the basis of pulmonary vascular resistance and oxygenation, and cellular damage on the basis of release of creatine kinase and aspartate aminotransferase. Neurologic assessment (score 0, normal; score 500, brain death) was done 6 hours after discontinuing cardiopulmonary bypass.

Results: Compared with animals undergoing cardiopulmonary bypass without ischemia (control animals), deep hypothermic circulatory arrest without modification of the reperfusate produced an oxidant injury (conjugated dienes increased 0.78 vs 1.71 absorbance (Abs) 240 nmol/L per 0.5 mL, $P < .001$ vs control animals), depressed cardiac output (6.0 vs 4.0 L/min, $P < .05$ vs control subjects), prolonged dopamine need ($P < .001$ vs control subjects), elevated pulmonary vascular resistance (74% vs 197%, $P < .05$ vs control subjects), reduced oxygenation ($P < .01$ vs control subjects), increased neurologic injury (56 vs 244, $P < .001$ vs control subjects), and increased release of creatine kinase (2695 vs 6974 U/L, $P < .05$ vs control subjects), aspartate aminotransferase (144 vs 229 U/L), and endothelin (1.02 vs 2.56 pg/mL, $P < .001$ vs control subjects). Conversely, the oxidant injury was markedly limited (conjugated dienes of 0.85 ± 0.09 Abs 240 nmol/L per 0.5 mL, $P < .001$ vs unmodified pump prime) with modification of cardiopulmonary bypass prime, resulting in increased cardiac output (5.1 ± 0.8 L/min), minimal dopamine need ($P < .001$ vs unmodified pump prime), no increase in pulmonary vascular resistance ($44\% \pm 31\%$, $P < .01$ vs unmodified pump prime) or endothelin levels (0.64 ± 0.15 pg/mL, $P < .001$ vs unmodified pump prime), complete recovery of oxygenation ($P < .01$ vs unmodified pump prime), reduced neurologic damage (144 ± 33 , $P < .05$ vs unmodified pump prime), and lower release of aspartate aminotransferase

(124 ± 23 U/L, $P < .05$ vs unmodified pump prime) and creatine kinase (3366 ± 918 , $P < .05$ vs unmodified pump prime).

Conclusions: A global reperfusion injury after deep hypothermic circulatory arrest was identified and changed. The injury is mediated by oxygen-derived free radicals, resulting in organ and endothelial dysfunction. Modification of global organ and endothelial damage is achieved by modifying the blood prime in the cardiopulmonary bypass circuit to deliver a controlled global reperfusate when reinstituting bypass.

Reperfusion injury produces functional, metabolic, and structural alterations following reintroduction of blood after temporary ischemia.¹⁻⁴ This damage after reperfusion is exaggerated when reperfusion takes place in an uncontrolled manner with unmodified (normal) blood.^{1,2} If the ischemia is prolonged, this can result in cellular damage, with impairment of end-organ function and an increase in morbidity and mortality. The cardiac surgeon is in the unique position to counteract this manner of reperfusion damage because the conditions of reperfusion and the composition of the reperfusate are under his or her direct control. Initial cardiac studies showed substantial injury reduction by modifying (controlling) the method of reperfusion after myocardial ischemia.^{1,2,5,6} Subsequently, similar underlying principles of controlled reperfusion were also found to limit reperfusion damage in the lung, kidney, and skeletal muscle.⁷⁻¹²

These focal studies made it clear that the principles of controlled reperfusion can be applied to the entire body. Global damage can result during efforts to repair certain congenital defects, as well as during adult cardiac surgery, because an interval of circulatory arrest may be needed to expedite surgical repair.^{13,14} If CPB is restarted in an uncontrolled fashion with unmodified blood, as is the usual clinical practice, the potential exists for a global reperfusion injury to occur in every organ. Such an injury would explain why generalized edema and multiple organ impairment are common after deep hypothermic circulatory arrest (DHCA).¹³⁻¹⁵ Studies in individual organs (heart, lung, kidney, and skeletal muscle), however, have shown that the fate of ischemic tissue is determined more by the method of reperfusion than by the duration of ischemia itself.^{1,2,5-12} It may therefore be possible to limit this global reperfusion injury after DHCA by applying the principles of controlled reperfusion pioneered in individual organs.

The aims of this study were to determine whether a global reperfusion injury occurs after DHCA and whether this injury could be avoided with controlled reperfusion by modifying the pump prime during the period of circulatory arrest to produce a modified global reperfusate.

Materials and Methods

Fifteen 2- to 3-month-old (29-35 kg) pigs were premedicated with 20 mg/kg ketamine and 0.25 mg/kg diazepam administered intra-

muscularly. Anesthesia was then achieved with inhaled isoflurane and fentanyl ($5 \mu\text{g/kg}$), and the lungs were ventilated with a volume ventilator. All animals received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research, and the "Guide for the Care and Use of Laboratory Animals," prepared by the National Academy of Sciences and published by the National Institutes of Health (publication No. 96-03, revised 1996).

The femoral artery and vein were cannulated to monitor arterial pressure for blood gas determinations and for intravenous infusions. After systemic heparinization (3 mg/kg), a thin-walled 18F to 20F, 52-cm-long venous cannula (Baxter Healthcare Corp, Irvine, Calif) was inserted into the femoral vein to the level of the right atrium, and a 14F to 16F thin-walled cannula was inserted into the femoral artery. A second 18F to 20F short venous cannula was placed into the right external jugular vein, and a Swan-Ganz catheter (Baxter) was placed into the pulmonary artery through the internal jugular vein to measure cardiac output. Arterial blood gases, electrolytes, and hemoglobin levels (Blood Gas System 288; Ciba Corning, Midfield, Mass) were measured every 15 to 30 minutes to ensure optimal levels. A heating-cooling blanket was placed below the pig, and a second pad was placed on the abdomen to maintain a constant core temperature of 37°C to 38°C when warm and 19°C during circulatory arrest. The cardiopulmonary bypass (CPB) circuit was heparinized, primed with packed red cells from donor pigs, and made normocalcemic. The hematocrit value was adjusted to 25% to 35% with donor blood and Plasmalyte solution (Baxter). A Baxter Univox Membrane Oxygenator (Baxter) was used, and the systemic flow rate was adjusted to approximately 100 to $125 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at full flow to maintain a continuously monitored aortic root pressure of 50 to 70 mm Hg.

Experimental Protocol

All animals were started on CPB with a fraction of inspired oxygen (Fio_2) of 100%, cooled to 19°C by using the alpha-stat strategy, and separated into 3 experimental groups. In 5 pigs CPB was continued without ischemia (control animals), whereas the other 10 animals underwent 90 minutes of DHCA. After DHCA, bypass was restarted at a pressure of 20 to 30 mm Hg and slowly increased to 50 to 70 mm Hg. All pigs were then rewarmed to 36°C or 37°C . The heart was defibrillated as needed at 25°C , and all animals were started on $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ dopamine once the core temperature reached 35°C . Dopamine was then either increased or weaned to keep systolic arterial pressure greater than 70 mm Hg and was discontinued only when arterial pressure was maintained at greater than 70 mm Hg for 5 minutes. Hemodynamic and pulmonary measurements were obtained before (baseline) and 60 minutes after CPB. Biochemical measurements were made before

TABLE 1. Modified global reperfusate

Component	Modified
Oxygen	Normoxia (PO ₂ 80-100 mm Hg)
Calcium	Citrate (Ca ⁺² 0.2-0.5 μg/dL)
Magnesium	MgSO ₄ (Mg ⁺² 2-4 mEq/L)
pH	pH-stat
Edema	Mannitol (12.5 g)
Na ⁺ /H ⁺ exchange inhibitor	HOE 642 (5 mg/kg)
WBC	WBC filtration CoBRA filter - bypass prime LG-6 filter - arterial line

CPB (baseline), at 15 minutes of rewarming (conjugated dienes [CD] and endothelin-1), and 4 hours after CPB was discontinued (creatine kinase [CK] and aspartate aminotransferase [AST]). After the hemodynamic measurements, protamine was given, and all cannulas were removed. Bleeding was controlled, the vessels were repaired, and all wounds were closed. The animals were then allowed to regain consciousness, were extubated, and underwent neurologic assessment approximately 4 hours later (6 hours after CPB). Fentanyl (0.2-0.3 μg/kg) was given as needed for postoperative pain but was held for at least 2 hours before the neurologic evaluation. The animals were then killed with 30 mg/kg phenobarbital and potassium chloride.

Experimental Groups

Control animals. Five pigs were cooled (19°C) and rewarmed by using the alpha-stat strategy and an FIO₂ of 100%, without any ischemia or period of circulatory arrest.

DHCA. After cooling to 19°C with the alpha-stat strategy and an FIO₂ of 100%, the other 10 pigs underwent 90 minutes of DHCA and were reperfused by using either the unmodified blood prime or a modified global reperfusate.

Unmodified CPB prime. CPB was reinstituted in 5 pigs without altering the pump blood bypass prime, oxygen concentration (100%), or pH strategy (alpha-stat), and the animals were rewarmed. This simulates the usual clinical practice.

Modified CPB prime. In the other 5 animals, the CPB prime was modified during the period of circulatory arrest to make a modified global reperfusate (Table 1). This included adding citrate to reduce the ionized calcium concentration to 0.2 to 0.5 μg/dL and magnesium sulfate to increase the ionized magnesium concentration to 2 to 4 mEq/L, reducing the oxygen tension (PO₂) to 80 to 100 mm Hg (normoxic strategy) and changing to a pH-stat management for the first 10 minutes of reperfusion, and adding a sodium-hydrogen ion exchange inhibitor (HOE 642, 5 mg/kg) and mannitol (12.5 g). The bypass prime was also leukocyte depleted by circulating the blood prime through 3 parallel conditioned blood reperfusion application (CoBRA) filters (Pall B-1328 filter; Pall Corporation, East Hills, NY) for 20 minutes during DHCA and an LG-6 high-flow leukocyte-depleting filter (Pall Corp, East Hills, NY) placed in the arterial line to continue white blood cell (WBC) removal during the entire period of rewarming.

Physiologic and Biochemical Determinations

Hemodynamic measurements. Hemodynamic measurements were made before and 60 minutes after discontinuing CPB. Car-

TABLE 2. Neurologic deficit scoring system

A. Central nerve function (0-100 points) Pupil size (0-5), light reflex (0-5), eye position (0-5), lid reflex (0-5), corneal reflex (0-5), ciliospinal reflex (0-5), oculocephalic reflex (0-5), auditory reflex (0-10), gag reflex (0-10), carinal reflex (0-15)
B. Respiration (0-100 points) Normal (0), hyperventilation (25), abnormal (50), absent (100)
C. Motor sensory function (0-100 points) Stretch reflex (0-25), motor response to pain (0-25), positioning (0-25), muscle tonus (0-25)
E. Level of consciousness (0-100 points) Normal (0), cloudy (30), delirium (45), stupor (60), coma (100)
F. Behavior (0-100 points) Drinking (0-15), chewing (0-15), sitting (0-15), standing (0-15), walking (0-40)

Total score is sum of all sections (0, normal; 500, brain death). Numbers in parentheses indicate scores for each parameter.

diac output was determined by means of duplicate injections of 4°C cold saline solution into the Swan-Ganz catheter. Cardiac output and pulmonary vascular resistance were calculated as previously described.^{7,10,12,16,17} Dopamine requirements are expressed as the total dose each animal received and calculated as the summation of the dose per minute multiplied by the total number of minutes at each dose.

Oxygenation. To assess pulmonary alveolar damage, the PO₂ was obtained on 100% oxygen before (baseline) and 1 hour after CPB by using a tidal volume of 12 to 15 mL/kg, and the ventilator rate was adjusted to maintain a PCO₂ of 36 to 44 mm Hg. Postoperative values are expressed as a percentage change from baseline, with each pig acting as its own control.

Conjugated dienes. CD, which are a marker of oxidant-mediated lipid peroxidation, were determined as previously described and expressed as the absorbance at a wave length of 240 nm/0.5 mL plasma.^{16,17}

CK and AST. Cellular injury was determined by measuring CK and AST activity with the UV-spectrophotometric method (Sigma Chemical Co, St Louis, Mo) and expressed as units per milliliter of plasma.

Endothelin-1. Vascular injury was determined by measuring endothelin levels with an Enzyme Immunometric Assay (ACE EIA Kit; Cayman Chemical Co, Ann Arbor, Mich) and expressed as picograms per milliliter of plasma.

Neurologic injury. Neurologic assessment was performed in all animals approximately 4 hours after wounds were closed, and the animals were extubated (6 hours after bypass). An adaption of neurologic deficit scoring described elsewhere was used, and a summary of the variables assessed are listed in Table 2.¹⁸ 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, whereas a score of 0 is normal. Neurologic deficit scoring was not blinded but was always agreed on by 2 members of the laboratory team. All animals were also kept under close supervision for any clinical evidence of seizure activity.

Figure 1. Oxidant injury, as measured by conjugated dienes production 15 minutes after initiating rewarming of CPB, is shown. * $P < .001$ versus control; ** $P < .001$ versus unmodified prime.

Figure 2. Vascular injury, as assessed by means of endothelin-1 (ET-1) release 15 minutes after initiating rewarming on CPB, is shown. * $P < .05$ versus control; ** $P < .001$ versus unmodified prime.

TABLE 3. Postoperative results

	Control (n = 5)	Unmodified prime (n = 5)	Modified prime (n = 5)	Statistical significance	
Physiologic					
Cardiac output (L/min)	6.0 ± 1.1	4.0 ± 0.3*	5.1 ± 0.8†	* <i>P</i> < .05	† <i>P</i> = NS
Total dopamine requirement (μg/kg)	325 ± 150	1620 ± 322*	323 ± 31†	* <i>P</i> < .001	† <i>P</i> < .001
Pulmonary vascular resistance (% increase)	74 ± 48	197 ± 49*	44 ± 21†	* <i>P</i> < .05	† <i>P</i> < .01
Oxygenation (% recovery)	95 ± 4	61 ± 8*	100 ± 13†	* <i>P</i> < .01	† <i>P</i> < .01
Biochemical					
Cd (Abs 240nm/L)	0.78 ± .12	1.71 ± 0.10*	0.85 ± 0.09†	* <i>P</i> < .001	† <i>P</i> < .001
ET-1 (pg/mL)	1.02 ± 0.05	2.56 ± 0.12*	0.64 ± 0.15†	* <i>P</i> < .001	† <i>P</i> < .001
CK (U/mL)	2695 ± 920	6974 ± 2064*	3366 ± 918†	* <i>P</i> < .05	† <i>P</i> < .05
AST (U/mL)	144 ± 25	229 ± 67*	124 ± 23†	* <i>P</i> = NS	† <i>P</i> < .05
Neurologic					
Deficit score (0-500)	56 ± 10	244 ± 34*	144 ± 33†	* <i>P</i> < .001	† <i>P</i> < .05

NS, Not significant.

* Control versus unmodified.

† Unmodified versus modified.

Statistics

Data were analyzed by using JMP V2.0 software (SAS Institute, Inc, Cary, NC) on a Macintosh IIVX computer (Apple Inc, Cupertino, Calif). The paired Student *t* test was used for comparison of variables among experimental groups. Group data are expressed as means ± SEM.

Results

Results are shown in Table 3 and Figures 1 to 3. No difference between groups was noted for baseline values of cardiac output (4.1 ± 0.1 L/min), pulmonary vascular resistance (385 ± 20 dynes $\cdot$ s⁻¹ $\cdot$ cm⁻¹ $\cdot$ m⁻²), Po₂ on 100% oxygen (521 ± 16 mm Hg), CD (0.62 ± 0.06 Abs 240 nm/L), endothelin-1 (0.80 ± 0.08 pg/mL), CK (263 ± 23 U/L), or AST (22 ± 9 U/L). There was also no difference between groups for the cooling (36 ± 1 minutes) or rewarming (69 ± 5 minutes) times.

Hemodynamic and Pulmonary Measurement

Postbypass cardiac output was excellent in both nonischemic (control) pigs and those receiving a modified reperfusion after DHCA. It was increased from baseline values, which probably represents the normal response as a result of higher endogenous catecholamines. These groups also required only low-dose dopamine to maintain adequate hemodynamics, and dopamine was discontinued by the time of cardiac output measurements in all animals. Likewise, the pulmonary vascular resistance was only mildly increased, and these animals sustained no significant alveolar damage because there was no change in postoperative oxygenation. In contrast, pigs receiving an unmodified reperfusion after DHCA had significantly higher dopamine requirements to maintain adequate hemodynamics. The cardiac output was lower despite 4 or 5 pigs still receiving dopamine at the time of the measurements. In addition, these pigs had markedly elevated pulmonary vascular resis-

tance and alveolar capillary damage, as demonstrated by a reduced recovery of postoperative oxygenation.

Biochemical Measurement

No increased CD or endothelin-1 efflux occurred in control (nonischemic) pigs or those undergoing DHCA with a modified reperfusate. Likewise, there was only a small increase in AST and a moderate increase in CK levels, indicating little tissue damage. In contrast, pigs undergoing DHCA with an unmodified reperfusate sustained a significant oxidant and vascular injury, with a marked increase in CD and endothelin-1 production. In addition, there was a substantial increase in both AST and CK levels, signifying significant tissue injury.

WBC Count

The prebypass total WBC count was $5.5 \pm 0.7 \times 10^3$ cells/mm³, which was not statistically different between groups. The WBC count was reduced with CPB to $3.5 \pm 0.4 \times 10^3$ cells/mm³ by the end of cooling but was once again not significantly different between groups. However, in pigs receiving modified reperfusion, the WBC count decreased to $0.2 \pm 0.1 \times 10^3$ cells/mm³ after filtering the bypass prime through the CoBRA filters. Five minutes after rewarming, the WBC count increased to only $1.6 \pm 0.6 \times 10^3$ cells/mm³ in the leukocyte-depleted pigs, whereas the WBC count increased to $4.5 \pm 0.9 \times 10^3$ cells/mm³ in the unfiltered nonischemic (control) and DHCA animals receiving an unmodified reperfusate. Four hours after bypass was discontinued, the WBC remained slightly lower in pigs receiving a modified reperfusate with leukocyte depletion ($6.3 \pm 1.1 \times 10^3$ cells/mm³) compared with that seen in control and unmodified pigs ($7.9 \pm 1.4 \times 10^3$ cells/mm³), but this was not statistically different ($P > .2$) and was higher than prebypass levels in all groups.

Neurologic Function

In nonischemic (control) pigs postoperative neurologic function was almost completely normal, with minimal impairment (Figure 3). However, pigs receiving an unmodified reperfusate after circulatory arrest had severe neurologic impairment, indicating substantial neurologic damage. They all had a severely depressed level of consciousness and were unable to stand or walk. In addition, 2 of 5 animals experienced generalized tonic clonic seizure activity. In contrast, the neurologic injury was substantially reduced in pigs receiving a modified reperfusate, resulting in significantly less neurologic impairment and no postoperative seizure activity. These pigs only had mild alterations in their levels of consciousness and were usually able to stand and walk.

Discussion

Clinical reports show that deep DHCA can increase morbidity and mortality by adversely changing postoperative

Figure 3. Neurologic injury 6 hours after discontinuing CPB, as assessed by means of neurologic deficit scoring, is shown. A score of 0 is defined as normal, and a score of 500 is defined as brain dead. Note the severe neurologic impairment in pigs undergoing circulatory arrest receiving an unmodified prime. In contrast, neurologic injury was significantly reduced in pigs receiving a modified prime after DHCA (see text for details). * $P < .001$ versus control; ** $P < .05$ versus unmodified prime.

organ function through ischemia and reperfusion damage, a presumably unavoidable consequence of DHCA.^{13-15,19,20} Our study confirmed that DHCA, followed by normal blood reperfusion (uncontrolled), produces vascular, tissue, and neurologic damage, with cardiac and pulmonary dysfunction. In contrast, controlled reperfusion with a modified reperfusate limits the formation of oxygen-derived free radicals, resulting in preservation of vascular, cardiac, pulmonary, and neurologic function.

We and others have shown that negative consequences of unmodified blood can be substantially avoided in isolated studies of the heart, lung, kidney, and skeletal muscle by controlling the initial period of reperfusion.^{1,2,5-12} Our global results are consistent with prior observations of the effects of controlled reperfusion in isolated organs. Controlled reperfusion has not previously been studied globally or locally in the brain. This study indicates that the principles of controlled reperfusion are effective in the brain because the neurologic score was significantly improved with a modified reperfusate, and no pig had seizures. A potential drawback is that we did not examine the brain histologically or look at biochemical markers of brain injury (ie, S-100 protein or neuron-specific enolase). Neurologic deficit scoring, however, directly correlates with pathologic, as well as biochemical, markers of injury.^{18,20-22} A similar correlation has also been seen with the production of oxygen-derived free radicals, which were substantially reduced with controlled reperfusion.

A second potential limitation is that the observation period was limited, with a neurologic assessment only performed 6 hours after bypass. However, leukocyte depletion alone has been shown to significantly reduce neurologic damage after DHCA in animals observed for up to 7 days.²²

Moreover, these investigations used a Pall LG-6 filter, which is relatively inefficient. In contrast, by using more efficient filters (as was done in the present study), as well as by modifying the constituents of the solution, vastly improved results in individual organs have been shown.^{2,4,7,11,12,23,24} We therefore believe that even in the absence of biochemical or histologic data, our findings confirm that controlled reperfusion significantly reduces neurologic injury. However, future studies will be needed to more precisely define the role of controlled reperfusion in limiting neurologic damage.

Each modification of the bypass prime was based on the principles established in individual organs.^{1,2,4,7,10,12} Calcium is one of the primary mediators of the reperfusion injury and may be the common pathway leading to cellular death.^{2,25} Reperfusion hypocalcemia was modulated in several ways. Citrate was added to directly reduce the ionized calcium levels. Magnesium levels were increased to both reduce intracellular calcium levels by inhibiting calcium entry across cellular membranes and to displace calcium from binding sites of the sarcolemmal membrane.^{4,26} Post-ischemic calcium entry is further limited because magnesium prevents the influx of sodium, which, during reperfusion, is exchanged for calcium. This Na^+/H^+ exchange mechanism was also addressed by providing a (HOE 642) sodium hydrogen ion exchange inhibitor. Ischemia and hypoxia produce intracellular acidosis from anaerobic glycolysis. The sodium hydrogen ion antiporter becomes activated in an attempt to correct this acidosis, leading to sodium overload.^{27,28} This sodium overload results in an intracellular calcium overload with reperfusion because sodium is exchanged for calcium. Exchange inhibitors, such as HOE 642, selectively block this antiporter, paradoxically preserving the intracellular acidosis but avoiding intracellular hypercalcemia and its associated injury.^{27,28}

The pH of the perfusate was changed to pH-stat through the initial period of reperfusion to further limit sodium hydrogen ion exchange and calcium deposition by means of the same mechanism. Mannitol was added to reduce edema and may also be beneficial because it is a potential oxygen radical scavenger.² The specific benefits of individual components is unknown because these factors interact. Each component was found to be important in individual organs, and for this reason, we applied these individual principles in an integrated way in constructing the global modified solution used in this study.^{1,2,4}

Although WBCs are involved mainly in the maintenance of the immune system, under certain pathologic conditions of altered physiology, they may cause damage to myocardial, pulmonary, or vascular tissue.^{4,7,23,24} 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.^{4,7,23,24,29} 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 in addition to releasing substances that are chemotactic for other neutrophils and macrophages, resulting in an amplification of the inflammatory response. Although WBCs can injure tissue by means of several mechanisms, numerous studies have demonstrated that the production of oxygen radicals is probably the major factor responsible for cellular damage.^{4,7,12,23,24,29} This explains the reduction in oxygen-derived free radical formation (lower CD) in pigs receiving leukocyte depletion, as well as the reduction in vascular damage (decreased endothelin-1), because activated WBCs cannot bind to the vascular endothelium if they are removed.

Leukocyte depletion was accomplished by using 2 different types of filters. First, a CoBRA filter was used during circulatory arrest to completely leukocyte deplete the bypass prime. The CoBRA filter is extremely efficient and removes greater than 99% of WBCs in a single pass.³⁰ It also attenuates the membrane attack complex, which can damage membranes directly. However, the CoBRA filter can only accommodate flows of up to 150 mL/min and becomes fully saturated after 500 to 750 mL of blood. We therefore, used 3 CoBRA filters in parallel to completely leukocyte deplete the prime. We strongly believe blood in the bypass prime should always be completely leukocyte depleted because oxygen-derived free radical formation is greatest with the initial reintroduction of oxygen and blood during reperfusion.^{7,23,24,29} An LG-6 filter was placed in the arterial line to continue the beneficial effects of leukocyte depletion. This filter can accommodate flows of up to 6 L/min for 1 to 2 hours before becoming saturated. It is less efficient in removing WBCs and instead removes neutrophils slowly over time. It also does not attenuate the membrane attack complex. The LG-6 filter, however, removes a significant number of WBCs at lower flow rates (1-2 L/min) because WBC removal is flow dependent. Therefore, an LG-6 filter is still very effective at reducing leukocytes in infants and small children. Use of inefficient WBC filters, coupled with leukocyte depletion during times that are less critical, probably explains why some investigators have failed to demonstrate a clinical advantage with WBC filtration, despite overwhelming experimental evidence to their benefit.^{7,12,23,24} Clearly, a more efficient in-line arterial filter that substantially reduces leukocyte counts in a single pass over the entire bypass run would be ideal. However, until such filters are commercially available, clinical improve-

ment will probably only be seen when WBC filters are used in a specific manner and directed at distinct events.

The management of blood pH during CPB remains a source of controversy and a subject of considerable recent clinical and laboratory investigations. Surgeons have sometimes taken adversarial approaches to pH management during DHCA, advocating either pH-stat or alpha-stat exclusively. Each strategy has its own advantage, and they may be integrated.^{13,14,19-22,31} pH-stat results in cerebral vasodilatation with more homogeneous brain perfusion, as well as more rapid cooling and rewarming. However, by leaving the cell in an acidic environment, pH-stat inhibits cellular metabolism during ischemia. An alternative may be pH-stat during cooling, followed by alpha-stat before circulatory arrest to delay evolving acidosis during ischemia. In contrast, alpha-stat causes cerebral vasoconstriction and reduced cerebral perfusion, leading to longer cooling and rewarming times. This may be particularly detrimental in the setting of increased pulmonary collaterals that compete for blood flow when perfusion pressure is fixed by CPB flow rate. The more alkaline pH of alpha-stat reduces pulmonary resistance and increases cerebral resistance, thereby increasing competition for blood flow between the cerebral and the pulmonary circulation. However, maintaining a more alkaline environment by means of late alpha-stat can help preserve and optimize enzymatic function in the face of ischemia. Therefore, a combined approach takes advantage of the strength while minimizing the weakness of each strategy.

We used an alpha-stat strategy in this study because it is commonly used in numerous institutions and is probably superior during the period of circulatory arrest because it preserves cellular metabolism during ischemia.^{13,14,19,20,22,31} A pH-stat strategy was used in the modified reperfusion group for the first 10 minutes of reperfusion to limit sodium hydrogen ion exchange and calcium deposition. Although pH-stat is probably also the optimal method for cooling and rewarming, it was only used for the first 10 minutes of rewarming to limit the differences between experimental groups. However, we believe that the ideal approach would be to integrate these 2 strategies by using pH-stat for longer intervals of cooling, rewarming, and reperfusion and to use alpha-stat just before the period of circulatory arrest. This may mimic hyperventilation before apnea, a helpful strategy.

A similar adversarial approach has occurred with oxygen strategy. Hyperoxia substantially increases an oxygen-mediated injury after hypoxia or ischemia.^{16,17,23,29} In contrast, maintaining normoxia (Po_2 of 80-100 mm Hg), rather than hyperoxia, during the initiation of CPB can substantially reduce oxidant damage and decrease the extent of tissue injury.^{16,23} These benefits coincide with the Po_2 -dependent nature of the injury because free radical production and myocyte injury after reoxygenation or reperfusion of isolated heart preparations are proportional to Po_2 .^{16,23} In clin-

ical practice, hyperoxic bypass is performed routinely but likely never needed because a Po_2 value of greater than 100 to 150 mm Hg confirms only a negligible increase in oxygen content and has been associated with an impairment in peripheral perfusion.^{23,32} The only exception is during a period of DHCA, when high oxygen levels have been shown to improve neuroprotection.^{19,33} However, these same studies found that initiating CPB after DHCA with a normoxic strategy significantly reduced formation of oxygen-derived free radicals. Therefore, as with pH strategy, an integrated approach making use of both normoxic and hyperoxic management probably provides the optimal therapy. This may provide balance through hyperoxia before arrest (DHCA) to raise cerebral Po_2 levels and slow depletion during ischemia: a preischemic preconditioning mechanism. Conversely, normoxic reperfusion can limit oxidant injury because flow is ensured by the CBP circuit.

Pigs in this study underwent 90 minutes of DHCA, which is significantly longer than what is commonly used in the clinical setting. However, these were normal, unstressed pigs. In contrast, most patients with cardiovascular disease are stressed by a variety of factors (ie, hypoxia, ischemia, acidosis, or pressure-volume overload) that may make them substantially less tolerant to the ischemic insult of DHCA. A similar evolution may occur in adults subjected to circulatory arrest (ie, aortic aneurysm procedures).

Even with a shorter period of circulatory arrest, any injury is unacceptable in the brain, which is extremely sensitive to ischemia and reperfusion. In addition, when combined with other necessary interventions (ie, cardioplegic arrest or prolonged CPB), a shorter period of circulatory arrest may still adversely affect the postoperative function in less susceptible organs.

Controlled reperfusion is not just changing the reperfusate but also the conditions of reperfusion.^{1,2,10} A lower initial reperfusion pressure (20-30 mm Hg) has been shown to reduce cellular damage in the heart, lung, and brain.^{2,4,10,14} When given before ischemia (pretreatment), numerous substances have also been found to lessen the reperfusion injury. For instance, sodium hydrogen ion exchange inhibitors (HOE 642) are much more effective if given before ischemia.^{27,28} In a sense, 100% oxygen is a pretreatment because it leaves the cell in an environment for maximal energy production and reduces the damage during ischemia. Using the same low perfusion pressure in both groups and, with the exception of 100% oxygen, avoiding pretreatment allowed us to examine the effects of the global reperfusate, without other compounding variables. However, lower initial pressure and pretreatment are both important adjuncts in limiting reperfusion damage and should always be used in concert with a modified reperfusate.

In conclusion, this study confirms that a reperfusion injury occurs after DHCA and demonstrates that it can be

significantly avoided by controlling the initial period of reperfusion with a global modified prime. Incorporating this modality will allow surgeons to reduce the damage after DHCA, leading to a significant reduction in morbidity and mortality and improved patient outcome.

We thank Ms Christina Green for her organizational and secretarial assistance.

References

- Allen BS, Okamoto F, Buckberg GD, Bugyi HI, Young H, Leaf J, et al. Studies of controlled reperfusion after ischemia XV: immediate functional recovery after 6 hours of regional ischemia by careful control of conditions of reperfusion and composition of reperfusate. *J Thorac Cardiovasc Surg.* 1986;92(suppl):621-35.
- Buckberg GD, Allen BS. Myocardial protection management during adult cardiac operations. In: Baue AE, Geha AS, Hammond GL, Laks H, Naunheim KS, editors. *Glenn's thoracic and cardiovascular surgery*. 6th ed. Stamford (CT): Appleton & Lange; 1995. p. 1653-87.
- Boyle Jr, Pohlman TH, Cornejo CJ, Verrier ED. Ischemia-reperfusion injury. *Ann Thorac Surg.* 1997;64:S24-30.
- Allen BS, Barth MJ, Ilbawi M. Pediatric myocardial protection: an overview. *Semin Thorac Cardiovasc Surg.* 2001;13:56-72.
- Buckberg GD. Strategies and logic of cardioplegic delivery to prevent, avoid and reverse ischemic and reperfusion damage. *J Thorac Cardiovasc Surg.* 1987;93:127-39.
- Kronon MT, Allen BS, Rahman SK, Wang T, Rayyab NA, Bolling KS, et al. Reducing postischemic reperfusion damage in neonates using a terminal warm substrate enriched blood cardioplegic reperfusate. *Ann Thorac Surg.* 2000;70:765-70.
- Halldorsson A, Kronon M, Allen BS, Bolling KS, Wang T, Rahman SK, et al. Controlled reperfusion after lung ischemia: implications for improved function after lung transplantation. *J Thorac Cardiovasc Surg.* 1998;115:415-25.
- Beyersdorf F, Matheis G, Kruger S, Hanselmann A, Freisleben HG, Zimmer G, et al. Avoiding reperfusion injury after limb revascularization: experimental observations and recommendations for clinical application. *J Vasc Surg.* 1989;9:757-66.
- Julia P, Haab F, Sabatier B, Fuzellier JF, Nochy D, Cambillau M, et al. Improvement of postischemic kidney function by reperfusion with a specifically developed solution (BT01). *Ann Vasc Surg.* 1995;9 Suppl:S81-8.
- Halldorsson AO, Kronon M, Allen BS, Rahman SK, Wang T. Lowering the pressure of the initial reperfusate reduces the reperfusion injury after pulmonary ischemia. *Ann Thorac Surg.* 2000;69:198-204.
- Allen BS, Hartz RS, Buckberg GD, Schuler J. Prevention of ischemic damage using controlled limb reperfusion. *J Card Surg.* 1998;13:224-7.
- Halldorsson A, Kronon M, Allen BS, Rahman SK, Wang T, Layland M, et al. Controlled reperfusion prevents pulmonary injury after 24 hours of lung preservation. *Ann Thorac Surg.* 1998;66:877-85.
- Williams WH, Davtyan HG, Drafnoda M. Hypothermia, cardiac surgery and cardiopulmonary bypass. In: Mora CT, Guyten RA, Rigatti RL, Finlayson DC, editors. *Cardiopulmonary bypass*. 1st ed. New York: Springer-Verlag; 1995. p. 40-54.
- Castaneda AR, Jonas RA, Mayer JE Jr, Hanley FL. Cardiopulmonary bypass, hypothermia, and circulatory arrest. In: Castaneda AR, Jonas RA, Mayer JE Jr, Hanley FL, editors. *Cardiac surgery of the neonate and infant*. Philadelphia: WB Saunders; 1994. p. 23-39.
- Bailey JM, Daly WL. Pediatric cardiopulmonary bypass. In: Mora CT, Guyten RA, Rigatti RL, Finlayson DC, editors. *Cardiopulmonary bypass*. 1st ed. New York: Springer-Verlag; 1995. p. 311-28.
- Buckberg GD. Studies of hypoxemic/reoxygenation injury: I. Linkage between cardiac function and oxidant damage. *J Thorac Cardiovasc Surg.* 1995;110:1164-70.
- Ihnen K, Morita K, Buckberg GD, Sherman MP, Young HH. Studies of hypoxemic/reoxygenation injury: without aortic clamping. III. Comparison of the magnitude of damage by hypoxemia/reoxygenation versus ischemia/reperfusion. *J Thorac Cardiovasc Surg.* 1995;110:1182-9.
- Forbess JM, Ibla JC, Lidov HGW, Cioffi MA, Hiramatsu T, Laussen P, et al. University of Wisconsin cerebroprotection in a piglet survival model of circulatory arrest. *Ann Thorac Surg.* 1995;60(suppl):S494-500.
- Nollert G, Nagashima M, Bucerius J, Shin'oka T, Lidov HG, du Plessis A, et al. Oxygenation strategy and neurologic damage after deep hypothermic circulatory arrest. II. Hypoxic versus free radical injury. *J Thorac Cardiovasc Surg.* 1999;117:1172-9.
- Bellinger DC, Wypij D, du Plessis AJ, Rappaport LA, Riviello J, Jonas RA, et al. Developmental and neurologic effects of alpha-stat versus pH-stat strategies for deep hypothermic cardiopulmonary bypass in infants. *J Thorac Cardiovasc Surg.* 2001;121:374-83.
- Priestley MA, Golden JA, O'Hara IB, McCann J, Kurth CD. Comparison of neurologic outcome after deep hypothermic circulatory arrest with alpha-stat and pH-stat cardiopulmonary bypass in newborn pigs. *J Thorac Cardiovasc Surg.* 2001;121:336-43.
- Rimpilainen J, Pokela M, Kiviluoma K, Anttila V, Vainionpää V, Hirvonen J, et al. Leukocyte filtration improves brain protection after a prolonged period of hypothermic circulatory arrest: a study in a chronic porcine model. *J Thorac Cardiovasc Surg.* 2000;120:1131-41.
- Allen BS, Ilbawi MN. Hypoxia, reoxygenation and the role of systemic leukodepletion in pediatric heart surgery. *Perfusion.* 2001;16(suppl):19-29.
- Kronon MT, Allen BS, Halldorsson A, Rahman SK, Wang T, Ilbawi M. L-arginine, prostaglandin, and white cell filtration equally improve myocardial protection in stressed neonatal hearts. *J Thorac Cardiovasc Surg.* 1999;118:665-73.
- Bolling KS, Kronon M, Allen BS, Ramon S, Wang T, Hartz R, et al. Myocardial protection in normal and hypoxically stressed neonatal hearts: the superiority of hypocalcemic versus normocalcemic blood cardioplegia. *J Thorac Cardiovasc Surg.* 1996;112:1193-201.
- Kronon M, Allen BS, Hernan J, Halldorsson A, Rahman SK, Buckberg GD, et al. Superiority of magnesium cardioplegia in neonatal myocardial protection. *Ann Thorac Surg.* 1999;68:2285-95.
- Karmazyn M. Comparative effects of Na^+/H^+ exchange inhibitors against cardiac injury produced by ischemia/reperfusion, hypoxia/reoxygenation and the calcium paradox. *J Cardiovasc Pharmacol.* 1993;21:172-8.
- Kim YL, Herijgers P, Laycock SK, Van Lommel A, Verbeken E, Flameng WJ. Na^+/H^+ exchange inhibition improves long-term myocardial preservation. *Ann Thorac Surg.* 1998;66:436-42.
- Allen BS, Rahman SK, Ilbawi M, Feinberg H, Bolling KS, Kronon M. The detrimental effects of cardiopulmonary bypass in cyanotic infants: preventing the reoxygenation injury. *Ann Thorac Surg.* 1997;64:1381-8.
- Patel MB, Kilgore KS, Ortolano GA, Gryboski CL, Qureshi MA, Marcovitz P, et al. Conditioned blood reperfusion during angioplasty (CoBRA) treatment of acute myocardial infarction. *Perfusion.* 2001;16(suppl):39-49.
- Jonas RA. Optimal pH strategy for hypothermic circulatory arrest. *J Thorac Cardiovasc Surg.* 2001;121:204-5.
- Joachimsson P, Sjöberg F, Forsman M, Johansson M, Casimir Ahn H, Rutberg H. Adverse effects of hyperoxemia during cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 1996;112:812-9.
- Nollert G, Nagashima M, Bucerius J, Shin'oka T, Jonas RA. Oxygenation strategy and neurologic damage after deep hypothermic circulatory arrest. I. Gaseous microemboli. *J Thorac Cardiovasc Surg.* 1999;117:1166-71.

Discussion

Dr Edward D. Verrier (Seattle, Wash). Which of the components makes the difference?

Dr Allen. I am often asked this question when I talk about our cardioplegic (*reperfusate*) solution, which is the basis for this approach. The answer is, every component is important. White cell filtration probably results in the biggest improvement, but every component makes a difference. Each acts in a specific way to limit the reperfusion injury. It is like asking what is the most important instrument in an orchestra. The answer is the one that is missing.