Proper Timing of Blood Cardioplegia in Infant Lambs: Superiority of a Multiple-Dose Regimen

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Background. **In the pediatric and infant age groups, it is unclear whether repeated infusions of blood cardioplegia solution during ischemic arrest are beneficial or detrimental when compared with a single-dose regimen.**

Methods. **Twenty lambs (aged 6 to 7 weeks) were placed on cardiopulmonary bypass. A miniature glass-tip electrode measured myocardial pH and hydrogen ion concentration, $[H^+]$, in the anterior wall. The aorta was clamped for 2 hours. Group S (n = 10) received a single dose of blood cardioplegia solution. Group M (n = 10) received multiple doses of blood cardioplegia solution at 20-minute intervals.**

Results. **The amount of $[H^+]$ generated during the cross-clamp period was greater in group S than in group M (39.2 ± 10.1 nmol/L versus 0.4 ± 1.4 nmol/L, $p < 0.008$). The percent increase in the time constant, tau, an index of diastolic relaxation, was more prolonged after cardiopulmonary bypass in group S when compared with group M (51.4% ± 2.8% versus 6.4% ± 3.0%, $p < 0.0001$). Similarly, the percent decrease in end systolic elastance, a measure of systolic contractility, was greater in group S after cardiopulmonary bypass when compared with group M (29.5% ± 1.4% versus 7.3% ± 1.3%, $p < 0.0001$).**

Conclusions. **In this infant lamb model, multiple doses of blood cardioplegia solution provided superior metabolic preservation and hemodynamic support after 2 hours of aortic clamping when compared with a single-dose regimen.**

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A wide variety of cardioplegia delivery techniques are currently used to facilitate intracardiac and extracardiac repairs of congenital heart defects [1]. The lack of a uniformly accepted policy regarding the administration of cardioplegia solution may, in part, reflect the confounding and inconsistent experimental data derived from animal models [2–8]. An important unresolved issue is whether or not multiple doses of cardioplegia solution provide an incremental benefit over an initial dose in nascent hearts. A multiple-dose regimen of cardioplegia solution has been associated with either improved or impaired recovery of ventricular function depending on the experimental protocol [2–4]. Several other studies have shown that repeated dosing of cardioplegia solution is actually detrimental when compared with a single-dose regimen [5–8]. The primary purpose of this study was to assess whether repeated dosing of blood cardioplegia solution is beneficial or deleterious to the preservation of myocardial metabolism and subsequent recovery of ventricular function in infant lambs.

Material and Methods

Experimental Model

Twenty infant lambs (11 to 13 kg) aged 6 to 7 weeks were housed and handled according to the "Guide for the Care and Use of Laboratory Animals" of the National Institutes of Health. The anesthetic agents included intramuscular ketamine (10 mg/kg), intramuscular xylazine (2 mg/kg), intravenous pancuronium (5 mg), and inhalational isoflurane (1% to 2%). Arterial pH was maintained between 7.30 to 7.40 by the appropriate adjustments of the ventilator and the administration of sodium bicarbonate when necessary. Systemic temperature was measured with a 9F rectal probe (model 24031, Medtronic, Minneapolis, MN).

A median sternotomy was performed. An 8- or 10-mm flow probe was placed around the innominate artery (Transonic Systems Inc, Ithaca, NY). A high-fidelity micromanometer 5F catheter (Millar Instruments, Inc, Houston, TX) was placed in the left ventricular (LV) apex for continual measurements of LV pressure and the maximum rate of increase of LV pressure (dP/dT). Two pairs of piezoelectric ultrasonic crystals were placed in the LV for orthogonal measurements of minor and major LV diameters (Sonometrics Inc, London, Ontario, Canada). The micromanometer and sonomicrometer signals were continuously recorded on a model TRXSenS digital ultrasonic measurement system. Myocardial pH and temperature were measured in the anterior wall of the LV with a miniature glass-tip electrode (Vascular Technology Inc, North Chelmsford, MA). A reference electrode was placed in the subcutaneous tissue [9, 10]. Myocardial pH and temperature data were continuously recorded on a computer. A pair of electrical pacing wires were placed on the right atrium and connected to a

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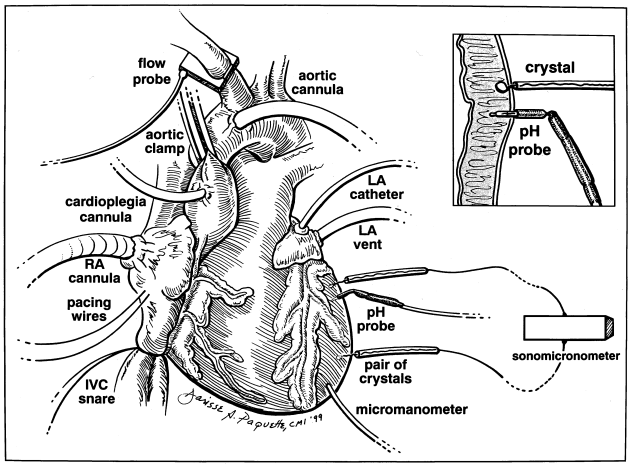

*Fig 1. Illustration of the experimental preparation. (*RA = *right atrium;* LA = *left atrium;* IVC = *inferior vena cava.)*

pacemaker (model 5530, Medtronic Inc). A snare was placed around the inferior vena cava and loosely threaded through a tourniquet. The ascending aorta was cannulated with a 12F cannula (DLP Inc, Grand Rapids, MI). The right atrium was cannulated with a 26F single-stage cannula. Figure 1 illustrates the experimental preparation.

After a 20-minute period of stabilization, baseline data were recorded. The lambs were then placed on cardiopulmonary bypass (CPB) at a flow rate of 150 mL/kg and cooled to a systemic temperature of 25°C. Arterial blood gases were managed according to the α-stat principle. An LV vent was placed through the base of the left atrium.

Experimental Protocol

After 20 minutes of systemic cooling the aorta was clamped for a period of 2 hours. The heart was arrested with an initial dose of blood cardioplegia solution (20 mL/kg) administered into the aortic root. The cardioplegia solution was composed of blood and a tromethamine-containing crystalloid solution (pH 7.45) mixed at a ratio of 4:1. The cardioplegia solution was delivered at a pressure of 80 to 100-mm Hg and a temperature of 6°C to 8°C. Cold saline solution was topically applied in the pericardial well during the entire cross-clamp period.

The animals were divided into two groups. Group S (n = 10) received a single dose of cardioplegia solution. Group M (n = 10) received multiple doses of cardioplegia solution (10 mL/kg) at 20-minute intervals during the remainder of the cross-clamp period. The initial dose of cardioplegia solution contained 24 mEq/L of KCl; additional doses consisted of 12 mEq/L of KCl. Systemic rewarming was commenced 30 minutes before release of the aortic clamp. The lambs were successfully weaned from CPB at a systemic temperature of 37°C. Inotropic support was not required. Repeat hemodynamic measurements were obtained 20 minutes after weaning from CPB.

Data Collection and Analysis

Myocardial pH was derived from electrode measurements of the electrical potential and a modification of the Nernst equation [9]. The pH electrodes were calibrated in pH buffers of 4.00 and 7.00 before and after each experiment. In each case the electrode drift was less than 0.05 pH units. Hydrogen ion concentration, $[H^+]$, was derived from the formula: $[H^+]$ = antilog (−pH). Accumulation of $[H^+]$ during the cross-clamp period was calculated by the difference between $[H^+]$ measured at the end of cross-clamp and $[H^+]$ measured at the beginning of cross-clamp.

Hemodynamic measurements were obtained before and after CPB. In each instance the heart rate was kept constant at 130 beats/min with atrial pacing. Measurements were obtained at a left atrial pressure of 5 cm H_2O. The maximal rate of increase of LV pressure (dP/dT) was determined by averaging data between 10 and 20 beats.

Tau, the time constant of relaxation, was derived from the micromanometer measurements of LV diastolic pressure during isovolumetric relaxation by the logarithmic method [11, 12].

Left ventricular pressure-volume loops were generated from the simultaneous sonomicrometer signals and the micromanometer readings of LV pressure. Different loading conditions were produced by tightening the inferior vena cava snare. Left ventricular volumes were estimated according to the ellipsoid formula [13].

The end-systolic elastance, E_{es}, was determined by calculating the slope of the pressure-volume loops at end-systole generated during inferior vena cava occlusion [13, 14].

The data are reported as the mean ± standard error of the mean. Repeated measures analysis of variance was used to identify differences between the various time periods and the two groups. Unpaired Student's *t* tests were used to compare differences between the two groups. The Bonferroni method was used to adjust for multiple comparisons. Statistical significance was achieved at a *p* less than 0.05.

Results

Myocardial temperature data are shown in Table 1. Myocardial temperatures were nearly identical before,

Table 1. Mean Myocardial Temperatures[a]

Group	On CPB	Cross-Clamp Time (min)[b] 30	60	90	120	Reperfusion (min)[b] 15	Off CPB
Group S Mean	37.8	14.9	14.6	14.5	14.9	35.8	35.7
Group S SEM	0.2	0.2	0.2	0.1	0.2	0.4	0.4
Group M Mean	37.6	15.3	14.7	14.4	14.5	34.8	36.1
Group M SEM	0.2	0.2	0.1	0.1	0.1	0.3	0.4
p Value	NS	NS	NS	NS	NS	NS	NS

[a] Myocardial temperatures are shown in degrees Celsius. [b] Data during the cross-clamp period are reported at 30-minute intervals. Reperfusion data are recorded 15 minutes after clamp removal.

CPB = cardiopulmonary bypass; NS = not significant.

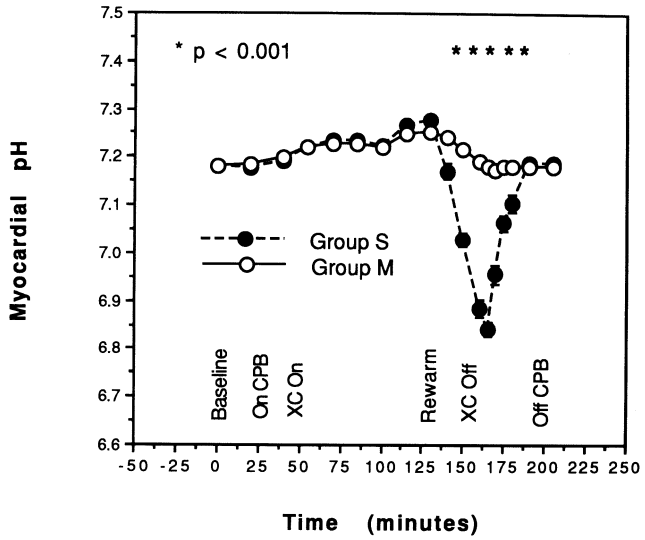

Fig 2. The time courses of myocardial pH for group S (solid circles) *and group M* (open circles) *are shown. During the initial phase of cardiopulmonary bypass* (CPB) *and the first 90 minutes of cross-clamp* (XC)*, myocardial pH values were similar between the two groups. However during the last 30 minutes of cross-clamp and during the reperfusion phase, myocardial pH values in group S were significantly lower than in group M. At the time of separation from bypass, myocardial pH values were identical in both groups.* *p < *0.001.*

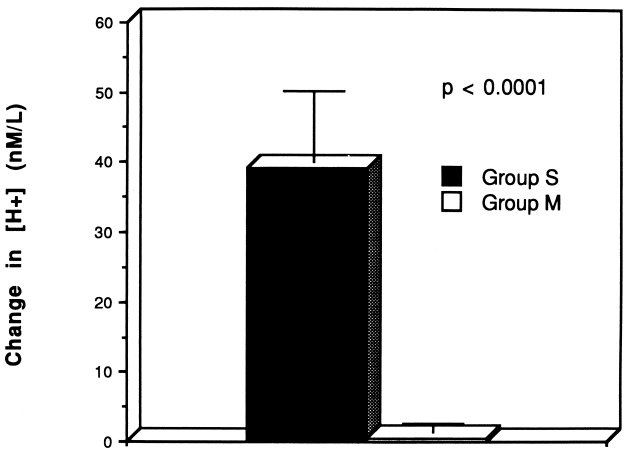

Fig 3. The accumulation of hydrogen ion, [H$^+$], during the cross-clamp interval was significantly greater in group S (closed bar) *than in group M* (open bar).

during, and after the cross-clamp period between the two groups.

Myocardial pH data for the two groups during the various stages of the experimental preparation are shown in Figure 2. The baseline pH data were identical in both groups: 7.18 ± 0.01. After the initiation of CPB and during the initial 90 minutes of aortic cross-clamping, there was a small increase in myocardial pH largely caused by systemic and myocardial cooling. Myocardial pH subsequently fell during the remainder of the cross-clamp period and the rewarming phase. However the fall in tissue pH was considerably greater during this time period in group S. At the time of weaning from CPB, myocardial pH had recovered in group S and was identical to group M: 7.18 ± 0.01.

There was a minimal accumulation of [H$^+$] during the cross-clamp period in group M (0.4 ± 1.4 nmol/L). In contrast there was a significant accumulation of [H$^+$] during the cross-clamp period in group S (39.2 ± 10.1 nmol/L, $p < 0.0001$). These data are illustrated in Figure 3.

Shown in Table 2 are the comparisons for tau, innominate artery flow, the maximum rate of increase of LV pressure, and E_{es} before and after CPB for the two groups. The rise in tau after CPB reached statistical significance in group S, but not in group M. There were statistically significant decreases in innominate flow, the maximum rate of increase of LV pressure, and E_{es} in group S. In comparison, the changes in group M were relatively mild.

The percent changes in tau and E_{es} for the two groups are illustrated in Figure 4. The percent increase in tau after CPB was significantly greater in group S (51.4% ± 2.8%) than in group M (6.4% ± 3.0%, $p < 0.0001$). Similarly the diminution in E_{es} after CPB was significantly greater in group S (29.5% ± 1.4%) than in group M (7.3% ± 1.3%, $p < 0.0001$).

Comment

Measurement of myocardial tissue pH has been shown to accurately detect and quantify the degree of ischemic damage during aortic cross-clamping in animal models and in humans [9, 10, 15–17]. However, with the exception of an isolated experimental study [18], the feasibility and potential benefits of pH monitoring have previously been limited to adult or mature hearts. The results of this study illustrate the importance of identifying and quantifying the degree of tissue acidosis in nascent myocardium. The multiple-dose regimen of blood cardioplegia solution minimized myocardial acid production during 2 hours of aortic clamping. This was associated with near complete recovery of both systolic and diastolic function.

Based on the assessment of myocardial pH, myocardial

Table 2. Hemodynamic Variables Before and After Cardiopulmonary Bypass

Variable	Time of Measurement	Group S	Group M
Tau (ms)	Pre-CPB	31.2 ± 0.5	30.9 ± 0.5
	Post-CPB	47.2 ± 0.9[a]	32.8 ± 0.8
Innominate flow	Pre-CPB	359.1 ± 26.7	367.4 ± 24.0
(mL/min)	Post-CPB	314.8 ± 23.8[a]	358.2 ± 23.6
d_p/d_t (mm Hg/s)	Pre-CPB	1113.6 ± 81.1	1042.2 ± 59.1
	Post-CPB	643.2 ± 120.6[a]	891.0 ± 101.4
E_{es} (mm Hg/mL)	Pre-CPB	10.5 ± 0.1	10.5 ± 0.1
	Post-CPB	7.4 ± 0.2[a]	9.7 ± 0.2

[a] $p < 0.05$ compared with the pre-CPB value.

CPB = cardiopulmonary bypass; d_p/d_t = maximum rate of increase of left ventricular pressure; E_{es} = end-systolic elastance.

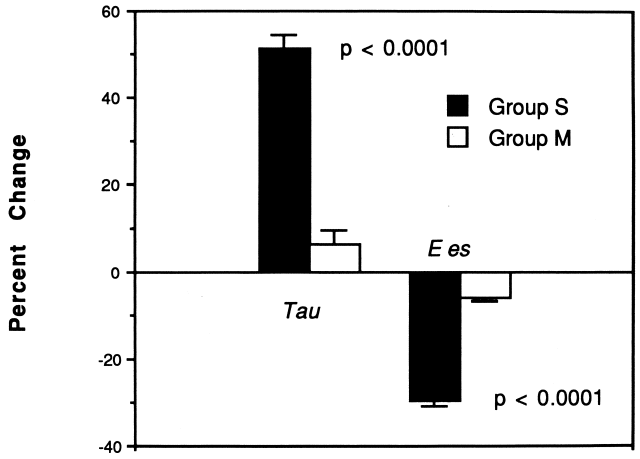

Fig 4. Shown are the percent changes in the post–cardiopulmonary bypass values of tau and the end-systolic elastance (E_{es}) *when compared with the pre–cardiopulmonary bypass values. The percent changes in tau and* E_{es} *were significantly greater in group S (*closed bars*) than in group M (*open bars*).*

protective strategies that use only a single dose of cardioplegia solution may provide sufficient metabolic protection for up to 90 minutes, However, the data from this study indicates that a single dose failed to sustain adequate metabolic support beyond 90 minutes of aortic clamping, resulting in progressive myocardial acidosis during the remainder of the cross-clamp interval and during the initial reperfusion period. The deleterious effects of persistent acidosis after release of the cross-clamp has also been documented in a clinical study [19]. The progression of tissue acidosis in the group S animals portended relatively poor systolic and diastolic performance after weaning from CPB.

Of particular interest is that the rapid decline in myocardial pH in group S was associated with the commencement of systemic rewarming. The fall in myocardial pH was not the result of regional rewarming as myocardial temperatures measured in the anterior wall were maintained at less than 15°C in both groups during the entire cross-clamp interval (Table 1). However, it is certainly possible that heterogeneous rewarming of the heart occurred during the latter stages of the cross-clamp interval producing regional temperature variations and suboptimal protection in the single-dose cardioplegia solution group.

The selection of blood as the cardioplegic vehicle in this study was based on its efficacy in limiting tissue acidosis in adult hearts [20–22]. The virtual absence of acid accumulation in group M illustrates the importance of buffering acid metabolites at regular intervals during extended periods of aortic clamping in immature hearts.

An important effect of multiple-dose cardioplegia solution on limiting myocardial acidosis is the washout of acid metabolites [23]. The infusion of cardioplegia solution at regular intervals in group M animals intermittently washed out the products of ischemic metabolism including H^+. In contrast, there was minimal, if any, washout of acid metabolites after the initial dose of cardioplegia solution in group S animals, resulting in the progressive buildup of H^+ during the cross-clamp interval.

In summary this study demonstrates that on-line monitoring of tissue pH in infant hearts provides a continual assessment of the adequacy of myocardial protection. Techniques that limit the amount of acid accumulation during the cross-clamp period, such as the repeated infusions of cardioplegia solution or the use of blood as the cardioplegic vehicle, are associated with better recovery of ventricular function. On-line measurement of myocardial pH during complex congenital heart repairs may lead to advances in clinical preservation techniques, resulting in enhanced ventricular performance and ultimately improved outcomes. Additional clinical studies are indicated to validate this strategy.

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INVITED COMMENTARY

Numerous controversies about the type and methods of delivery of cardioplegic solutions continue to prevail, despite a voluminous literature of experimental studies published over the past 15 years. This fact alone underscores the need for more sensitive tools with which reliable online assessments of the adequacy of myocardial protection can be obtained. This experimental study by Warner and colleagues demonstrates the value of employing an online metabolic monitor to settle, at least experimentally, an important controversy related to the merits of single versus multidose cardioplegia in the infant heart. Prolonged aortic clamping of the infant heart resulted in progressive myocardial tissue acidosis in the anterior left ventricular wall when cardioplegia was administered through a single dose; it also resulted in post-reperfusion cardiac dysfunction. In contrast, multidose administration of the same solution completely prevented acidosis in that wall, and preserved post-reperfusion cardiac function. One of the limitations of this study, however, is the use of a single electrode, placed in the anterior wall, with the assumption that the distribution of the cardioplegic solution in the heart was homogeneous, and that the pH in the anterior wall was representative of pH in the other walls. Clinical studies, which employed the electrode described in this study intraoperatively in adult patients, have shown a variable and an unpredictable heterogeneity in the distribution of the cardioplegic solution administered during the period of aortic clamping. This heterogeneity is manifested by significant and often marked differences between the myocardial pH levels observed in the anterior and posterior walls of the left ventricle, with the posterior wall demonstrating increased vulnerability to the onset of acidosis. These differences, which have been observed even in the absence of coronary artery disease, underscore the importance of monitoring myocardial pH in both the anterior and the posterior walls of the left ventricle.

In addition to demonstrating the value of myocardial pH measurement in the assessment of the adequacy of myocardial protection, this study is one of the first clinically-relevant experimental studies which show the adverse impact of myocardial acidosis during the period of aortic clamping on post-reperfusion cardiac function. There is now compelling evidence from myocyte culture studies that acidosis is a primary trigger for apoptosis. This finding, together with the adverse impact of myocardial tissue acidosis demonstrated in this study, suggest that future myocardial protection strategies should be based on the prevention of regional myocardial acidosis—an approach which will be aided by the development of tools for the on-line assessment of myocardial pH.

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