Myocardial Protection and Vascular Biology

All-Blood (Miniplegia) Versus Dilute Cardioplegia in Experimental Surgical Revascularization of Evolving Infarction

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Background—The advantages of blood cardioplegia include the oxygen-carrying capacity, superior oncotic and buffering properties, and endogenous antioxidants contained in blood. However, the partial dilution of blood in 4:1 (blood:crystalloid) cardioplegic solutions may nullify these advantages and progressively dilute blood during continuous retrograde delivery. This study tested the hypothesis that all-blood (66:1) cardioplegia provides superior myocardial protection compared with dilute (4:1) cardioplegia delivered in a continuous retrograde modality during surgical reperfusion of evolving myocardial infarction.

Methods and Results—After 60 minutes of left anterior descending coronary artery (LAD) occlusion, anesthetized canines were placed on cardiopulmonary bypass and randomized to either all-blood cardioplegia (AB group) or dilute blood cardioplegia (Dil group). After cross clamping, arrest was induced with 5 minutes of tepid (30°C) antegrade potassium all-blood or dilute blood cardioplegia and maintained with tepid retrograde coronary sinus cardioplegia for a total of 1 hour. The LAD was released after 30 minutes of arrest, simulating revascularization. The cardioplegia hematocrit for the Dil group was lower than that for the AB group (7±1% versus 12±2%, P<0.05); at the end of bypass, systemic hematocrit was lower in the Dil group than in the AB group (15±1% versus 20±1%, P<0.05). Infarct size (triphenyltetrazolium chloride staining) was comparable between the AB and Dil groups (29.6±2.9% versus 30.3±3.9% of area at risk), and there was no difference in area-at-risk myocardium systolic shortening (by sonomicrometry, −0.3±1% versus −0.4±1%). Tissue edema after bypass tended to be greater in the Dil group compared with the AB group in the heart (82±0% versus 81±1%), lung (79±1% versus 78±1%), liver (75±1% versus 74±0%), and skeletal muscle (76±1% versus 73±2%) and was significantly greater in the duodenum (80±1% versus 79±1%, P<0.05) and kidney (82±1% versus 79±1%, P<0.05). Postexperimental endothelial function (relaxation of acetylcholine) was impaired in LADs of the AB group versus the Dil group (59±6% versus 77±5%, P<0.05).

Conclusions—Both all-blood cardioplegia and dilute cardioplegia have disadvantages, but these do not have an impact on the pathogenesis of infarct size or recovery of regional contractile function. (Circulation. 2001;104[suppl I]:I-296-I-302.)

Key Words: cardioplegia  endothelium  infarction  surgery  edema

In the late 1970s and early 1980s, studies by Dr Buckberg’s laboratory (Follette and colleagues1–3) suggested that blood provided the best vehicle for delivery of cardioplegia in myocardium potentially injured by antecedent ischemia. The use of blood was based on its superior oxygen-carrying capacity, better osmotic properties and buffers, endogenous nutrients, and antioxidants compared with its crystalloid counterpart. The original formulation of blood cardioplegia was hemodiluted in a ratio of 4 parts blood to 1 part crystalloid, in part to avoid rouleau formation. In addition, moderate hemodilution would theoretically reduce the concentration of inflammatory mediators (ie, tumor necrosis factor [TNF]-α and interleukins) and cells (polymorphonuclear leukocytes [PMNs]), which are stimulated during both cardiopulmonary bypass and myocardial ischemia.4 These inflammatory mediators and cells have been shown to participate in the systemic inflammatory response to cardiopulmonary bypass and procedures requiring hypothermic circulatory arrest, causing multiorgan dysfunction.4

In contrast to the theoretical advantages of moderately hemodiluted blood cardioplegia, Menasché and colleagues5,6 suggested that undiluted blood cardioplegia or “miniplegia” would retain all the advantages of blood cardioplegia without the potential disadvantages of hemodilution. Miniplegia simplifies the formulation of blood cardioplegia by using a minimum amount of crystalloid additive, which would theoretically use more fully the endogenous properties of blood while arresting the heart and attenuating calcium-related...
abnormalities with adjunct magnesium, rather than by treat-
ing the myocardium with a complex array of buffers, calcium-chelating agents, and metabolic additives. In addi-
tion, minimizing the degree of hemodilution ostensibly al-

ows blood to exert its endogenous protective effects.6 How-

ever, the physiological benefits of moderately diluted versus

 minimally diluted blood cardioplegia have not yet been

elucidated.

Accordingly, the present study was designed to compare

the effect of moderately diluted blood cardioplegia (4 blood:1

crystalloid) with all-blood cardioplegia (ie, miniplegia; 66

blood:1 crystalloid) on myocardial and vascular protection as

well as tissue edema in multiple organs in an experimental

model of injured myocardium created by acute regional

coronary artery occlusion and surgical reperfusion. Blood

cardioplegia was administered by using antegrade arrest

followed by continuous retrograde delivery. This modality of
delivering cardioplegia represents the worst-case scenario, in

which high volumes of cardioplegia are delivered, potentially

expressing the physiological effects of cumulative hemodilu-
tion by using the 4:1 blood cardioplegic strategy.

Methods

Surgical Procedure

The dogs were handled in compliance with the Guide for the Care

and Use of Laboratory Animals published by the National Institutes

of health (publication No. [NIH] 85-23, revised 1985). The Institu-
tional Animal Care and Use Committee of Emory University

approved the study protocol.

Twenty microintra-aortic mongrel dogs of either sex, weighing 25

to 34 kg, were premedicated with morphine sulfate (4 mg/kg), and

anesthesia was induced with sodium thiopental (25 mg/kg). After

droterochondral intubation, anesthesia was maintained by using a

mixture of diazepam (0.03 mg · kg⁻¹ · min⁻¹) and fentanyl citrate

(0.03 g · kg⁻¹ · min⁻¹) administered via continuous intravenous

infusion. The dogs were ventilated with a volume-cycled respirator

with oxygen-enriched room air. Ventilatory variables were adjusted
to maintain arterial oxygen tension of >100 mm Hg, P CO₂ between

30 and 40 mm Hg, and pH in the range of 7.35 to 7.45. Sodium

bicarbonate was administered intravenously to treat acidemia. Body

temperature was maintained with a heating blanket. The right femoral

artery was cannulated for arterial blood sampling and pressure monitoring.

After median sternotomy, the azygos vein was ligated, and a

pericardial cradle was created to suspend the heart. Solid-state

catheter-tipped pressure transducers (Millar Instruments) were intro-
duced into the ascending aorta via the right main pulmonary artery and into the

left ventricle via the apex. Piezoelectric crystals (Tricon, Inc)

were placed into the myocardium in the distribution of the left

anterior descending coronary artery (LAD) to measure circumferen-
tial shortening. The LAD was dissected free just distal to the first
diagonal and looped with a 2-0 silk suture for later occlusion.

After systemic anticoagulation with sodium heparin (300 U/kg),

the left subclavian artery was cannulated for arterial perfusion.
The superior and inferior venae cavae were transatrially cannulated, and
the cannula tips were kept in the atrium until the institution of

cardiopulmonary bypass to avoid hemodynamic interference. Base-

line data (both steady-state and gradual preload reduction) were

taken for hemodynamic and cardiodynamic analysis.

Experimental Protocol

Animals were randomized to receive either moderately diluted (4

parts blood:1 part crystalloid) cardioplegia (Dil group) or all-blood

(66 parts blood:1 part crystalloid) cardioplegia (AB group). The

crystalloid component of the cardioplegia was a Krebs-Henseleit

buffer with the following composition (in mmol/L): NaCl 118, KCl

4.7, KH₂PO₄, 1.2, MgSO₄, 1.2, CaCl₂, 2.5, NaHCO₃, 12.5, and glucose

11, with pH 7.4. This crystalloid was used instead of saline or other

electrolyte solution as a crystalloid component of cardioplegia to

prevent dilution of key electrolytes (Ca²⁺, K⁺, and Mg²⁺) in the Dil

group, which would otherwise confound the interpretation of such

results as regional function.

After 60 minutes of LAD ischemia, the canulaus were advanced, and
cardiopulmonary bypass was initiated. The cardiopulmonary

bypass circuit consisted of a membrane oxygenator (Cobe Cardio-

vascular) primed with 1.5 L of Hextend (Hespan, DuPont Phar-

aceutical). A double lumen aortic root cannula (DLP, Inc) was

placed in the ascending aorta for cardioplegia delivery and pressure

monitoring. Blood was passed through a Cobe 43-μm pore filter.

Myocardial temperature probes were inserted in the LAD and

posterior surfaces of the heart. Heparin was supplemented every 90

minutes, with 150 U/kg, to maintain activated clotting times of

>400 seconds. The left ventricle was vented via gravity drainage. Also, a

retrograde cardioplegia delivery cannula was inserted into the

coronary sinus. The antegrade cardioplegia was delivered for 5

minutes at 30°C with a potassium concentration of ~20 mmol/L,

after which the cardiopleic delivery was diverted to retrograde delivery for

55 minutes at 30°C with a potassium concentration of 10 mmol/L.
The remaining 5 minutes was delivered antegradely at 37°C with 20

mmol/L potassium. Cardioplegia was delivered at flows of 200 mL/min antegradely and to

a pressure of 35 to 45 mm Hg during retrograde infusion by using the

Myocardial Protection System (Quest Medical Inc). The occlud-
ing LAD ligature was released 30 minutes after the beginning of

cardioplegia to simulate surgical revascularization. The aortic cross-

clamp was released immediately after completion of the terminal

infusion, and the heart was allowed to beat empty for 30 minutes.

Ventricular fibrillation was counteracted with DC cardioversion of

10 to 20 J. After 30 minutes in the empty beating state, cardiopul-

monary bypass was discontinued, and the heart was allowed to beat

fully for 30 minutes.

Data Acquisition and Analysis

A microcomputer system (IBM-PC) with an analog-to-digital con-

verter (model DT2801A, Data Translation) was used to record

hemodynamic and cardiodynamic data at a sample rate of 250 Hz.

The data were stored and analyzed by using SPECTRUM cardio-

vascular acquisition and analysis software (Wake Forest University).

Data were acquired before LAD occlusion (baseline), after 45

minutes of ischemia, after 30 minutes of empty beating on bypass, and

after 30 minutes of beating off bypass. Averages of the

hemodynamic and cardiodynamic data were calculated from no less

than 10 cardiac cycles. Segmental work, percent systolic shortening,

and segmental stiffness were determined as previously described.7 8

Determination of AAR and Infarct Size

At the end of the experiment, the heart was excised, the LAD was

religated, and the myocardial area at risk (AAR) was determined by

injecting Unisperse blue dye (Ciba-Geigy). Infarct size was deter-

mined by incubating transverse slices of the left ventricle in 1%

triphenyltetrazolium chloride (Sigma Chemical Co) at 37°C as

previously described.9 Infarct size was expressed as a percentage of

the AAR.

Determination of Tissue Water

Postexperimental tissue samples of ~0.3 g were taken from the heart

(nonischemic, AAR), brain, lung, liver, duodenum, muscle, and

kidney. The tissue was weighed and desiccated for 48 hours at

80°C. Percent water was calculated as (wt weight−dry weight)/(wt

weight)×100.

MPO Activity

Tissue samples of ~0.4 g from ischemic, necrotic, and nonischemic

myocardium were taken for spectrophotometric analysis of myelo-

peroxidase (MPO) activity as an indicator of neutrophil accumula-
tion. MPO was described as the rate of H2O2 degradation–induced color change per minute per 100 mg tissue.

Plasma CK
Spectrophotometric analysis (CK-10 kit, Sigma Diagnostics) was performed for creatine kinase (CK) activity and protein concentration (Sigma Diagnostics) from arterial blood samples. CK was expressed as international units per gram of protein.

Postischemic Endothelium Function
Neutrophil Adherence to Coronary Endothelium (Basal Endothelial Function)
At baseline, arterial blood was withdrawn for neutrophil (PMN) isolation by using the Ficoll-Paque density gradient technique. Unstimulated fluorescently labeled PMNs were used to assess basal endothelial function by examining their adherence to the endothelial surface of postexperimental canine epicardial coronary arteries, as previously described by Zhao et al.10 Adherent neutrophils were counted under epifluorescence microscopy (490-nm excitation, 504-nm emission).

Agonist-Stimulated Vasorelaxation Responses
Ischemic/reperfused and nonischemic coronary arteries were isolated, divided into 4- to 5-mm-wide rings, and placed in buffer-perfused organ chambers filled with Krebs-Henseleit solution at 37°C. Indomethacin was added to the buffer solution to prevent the vasoactive effects of prostanoids. The vessel was preconstricted with vasodilator sodium nitroprusside.

TABLE 1. Hemodynamic and Regional Cardiodynamic Variables and Plasma CK Activity

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Ischemia</th>
<th>R30</th>
<th>R60</th>
</tr>
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<tbody>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>109±13</td>
<td>146±11</td>
<td>131±7</td>
<td>136±6</td>
</tr>
<tr>
<td>Dil</td>
<td>91±5</td>
<td>126±13</td>
<td>122±6</td>
<td>124.7±5</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>86±3</td>
<td>81±4</td>
<td>69±3</td>
<td>65.5±4</td>
</tr>
<tr>
<td>Dil</td>
<td>72±4</td>
<td>79±5</td>
<td>74±4</td>
<td>62.9±3</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>12.9±1.0</td>
<td>14.7±1.0</td>
<td>16.7±1.0</td>
<td>14.2±1.0</td>
</tr>
<tr>
<td>Dil</td>
<td>12.2±1.0</td>
<td>16.3±1.0</td>
<td>17.0±2.0</td>
<td>12.9±1.0</td>
</tr>
<tr>
<td>SS%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>22±3</td>
<td>−5.0±0.8*</td>
<td>0.8±0.9</td>
<td>−0.3±1.0</td>
</tr>
<tr>
<td>Dil</td>
<td>16±3</td>
<td>−6.0±1.0*</td>
<td>2.0±0.2</td>
<td>−0.4±1.0</td>
</tr>
<tr>
<td>CK, U/g protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1.2±0.3</td>
<td>2.0±0.2</td>
<td>24±2*</td>
<td>31±4*</td>
</tr>
<tr>
<td>Dil</td>
<td>2.0±0.2</td>
<td>2.5±0.5</td>
<td>28±5*</td>
<td>31±5</td>
</tr>
</tbody>
</table>

*P<0.05 vs previous time value.

Results

Hemodynamics
Hemodynamic measurements are summarized in Table 1. At baseline, both groups had similar values for heart rate. Heart rate increased during ischemia and reperfusion, but there were no statistical differences. Mean arterial pressure decreased during the course of the experiment but with no statistical differences between groups. Left ventricular end-diastolic pressure was comparable between groups at any given time and did not change after reperfusion.

Regional systolic shortening was comparable between groups at baseline (Table 1). Active segmental contraction was converted to comparable levels of dyskinesis during coronary occlusion. There was no significant recovery of systolic shortening in the LAD myocardium after 30 minutes of reperfusion (vented bypass) or after 60 minutes of reperfusion (intact circulation off pump).

The total volume of blood cardioplegia administered was not significantly different between groups (6.0±0.7 L for the AB group versus 6.7±0.7 L for the Dil group). However, the total volume of crystalloid additive delivered to the Dil group was 19-fold greater than that delivered to the AB group (1668±38 versus 90±31 mL, respectively; P<0.05). Accordingly, the hematocrit of the cardioplegia in the AB group was significantly higher than that in the Dil group after completion of retrograde and terminal “hot-shot” deliveries (Table 2). All other compositional variables of the cardioplegic solution were comparable between the 2 groups.

Statistical Analysis
Data were analyzed by using Sigma Stat (SPSS Science, Inc) software. Time-related differences and group-time interactions for longitudinal data were analyzed by using 2-way ANOVA for repeated measures adjusted for baseline values. The Tukey post hoc test was used to locate the source of difference once these were assigned. Single-event nonrepeated variables were compared between the groups at the same base cardioplegic solution (ie, AB versus Dil groups) with the use of a standard t test. A value of P<0.05 was considered significant. All values are expressed as mean±SEM.
addition, systemic hematocrit was comparable after initiation of bypass in the Dil and AB groups (24.6% versus 21.6%, respectively) but decreased significantly after completion of cardioplegia delivery (15.6% for Dil group versus 20.6% for AB group, \( P < 0.05 \)).

Infarct Size

LAD occlusion placed a comparable amount of left ventricular myocardium at risk between groups (48.6% in the AB group versus 46.5% in the Dil group). Infarct size in the AB group (29.6% of AAR) was not significantly different from that in the Dil group (30.3% of AAR).

Plasma CK Activity

Plasma CK activity was similar between groups at baseline (Table 1). Plasma CK activity showed a modest and comparable increase in both groups throughout the reperfusion period. There were no significant differences between groups at any time point.

Tissue Water

Tissue water content is shown as a percentage in Table 3. Tissue water content in AAR and in nonischemic left ventricular myocardium was comparable between groups, and tissue water content in the AAR in both groups was greater than that observed in control myocardium (78.2% vs 6.5%, \( n = 8 \) not exposed to ischemia/reperfusion or cardiopulmonary bypass. Water content was not significantly different between groups in the brain, lung, liver, or gastrocnemius skeletal muscle. However, the duodenum and kidney showed a higher tissue water content in the Dil group compared with the AB group.

Myocardial MPO Activity

There were no statistical differences in MPO activity between the Dil and AB groups (Figure 1).

Postischemic Basal Endothelial Function

Healthy endothelium attenuates adherence of unstimulated neutrophils largely through the tonic release of NO. Dysfunctional endothelium, on the other hand, is associated with adherence of unstimulated neutrophils roughly in proportion to the degree of injury sustained. In the AB group, compared with the Dil group, neutrophil adherence to postexperimental coronary artery endothelium was significantly greater (by 32%) in the ischemic/reperfused LAD (Figure 2). In addition, neutrophil adherence in the nonischemic left circumflex coronary artery (LCx) region was significantly greater in the AB group than in the Dil group. Therefore, the AB group was associated with greater adherence of unstimulated neutrophils in both the ischemic/reperfused LAD and the nonischemic LCx, suggesting damage to the coronary artery endothelium.

Vascular Relaxation in Postexperimental Coronary Arteries

Baseline tension in coronary artery rings ranged from 2.8±0.1 to 3.1±0.1 g, with no group differences. Contraction

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**TABLE 2. Cardioplegia Variables**

<table>
<thead>
<tr>
<th></th>
<th>Induction</th>
<th>Continuous Retrograde</th>
<th>Terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium, mEq/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>28±1</td>
<td>16±1</td>
<td>29±2</td>
</tr>
<tr>
<td>Dil</td>
<td>24±2</td>
<td>14±1</td>
<td>25±2</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>7.32±0.02</td>
<td>7.43±0.03</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>Dil</td>
<td>7.37±0.02</td>
<td>7.48±0.02</td>
<td>7.48±0.02</td>
</tr>
<tr>
<td>Hct, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>14.0±1.0</td>
<td>14.3±1.2*</td>
<td>11.7±1.5*</td>
</tr>
<tr>
<td>Dil</td>
<td>10.9±1.9</td>
<td>8.3±1.1</td>
<td>6.8±1.4</td>
</tr>
<tr>
<td>Mg²⁺, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>0.7±0.2</td>
<td>1.4±0.2</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Dil</td>
<td>0.4±0.1</td>
<td>1.4±0.1</td>
<td>0.5±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \( *P < 0.05 \) vs corresponding value for Dil group.

**TABLE 3. Tissue Water Content**

<table>
<thead>
<tr>
<th>Tissue Water Content, %</th>
<th>AB Group</th>
<th>Dil Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart, nonischemic</td>
<td>81.1±0.6</td>
<td>82.2±0.2</td>
</tr>
<tr>
<td>Heart, ischemic</td>
<td>81.1±0.8</td>
<td>82.1±0.3</td>
</tr>
<tr>
<td>Brain</td>
<td>76.1±0.7</td>
<td>76.6±0.9</td>
</tr>
<tr>
<td>Lung</td>
<td>78.5±0.8</td>
<td>79.3±1.1</td>
</tr>
<tr>
<td>Liver</td>
<td>74.2±0.4</td>
<td>75.3±0.6</td>
</tr>
<tr>
<td>Duodenum</td>
<td>77.8±0.4</td>
<td>79.7±0.5*</td>
</tr>
<tr>
<td>Kidney</td>
<td>79.1±0.9</td>
<td>81.7±0.7*</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>73.5±1.7</td>
<td>75.7±0.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \( *P < 0.05 \) vs AB group.

**Figure 1.** Neutrophil accumulation (MPO activity) in myocardium from nonischemic subepicardial (N-Epi), nonischemic subendocardial (N-Endo), ischemic subepicardial (I-Epi), and ischemic subendocardial (I-Endo) zones. There were no significant differences between groups or zones.

**Figure 2.** Postexperimental function of coronary artery endothelium assessed by adherence of unstimulated neutrophils. Note that adherence of neutrophils is less in the dilute group for both LAD and nonischemic \( *P < 0.05 \) vs same artery in dilute group.
responses to U46619 ranged from 7.3±0.3 to 7.5±0.3 g, with no group differences. Stepwise relaxation responses were observed to incremental concentrations of ACh. Maximum relaxation responses to ACh was impaired in the AB group compared with the Dil group in the LAD (59±6% versus 77±5%, respectively; *P*, 0.05 Figure 3A) and the LCx (67±8% versus 84±6%, respectively; *P*, 0.5, Figure 4A). Smooth muscle relaxation responses to incremental concentrations of sodium nitroprusside were comparable between the AB and Dil groups for the LAD (maximum 84±5% versus 77±8%, respectively) but were significantly lower in the AB group in the LCx (maximum 77±5% versus 95±7%, respectively; *P*, 0.05) (Figure 4B).

Discussion

In the present study, we investigated the use of all-blood (66:1) cardioplegia compared with a popular, more diluted form (4:1) of blood cardioplegia in an acute ischemia/reperfusion model in which the AAR is vulnerable to ischemic/reperfusion injury. A tepid continuous retrograde delivery modality was used to simulate a clinical option for cardioplegia delivery used for coronary revascularization surgery, albeit not a universally used modality. After 1 hour of continuous sequential antegrade and retrograde delivery, there was a significantly greater volume of crystalloid additive administered in the Dil group, with a progressive dilution of the hematocrit of systemic blood and the cardioplegic solution. Accordingly, the Dil group was associated with greater peripheral edema in select organs (duodenum and kidney) and with no significant edema in other organ systems. On the other hand, the AB group was associated with moderate coronary artery endothelial dysfunction assessed by 2 complementary methods. Vasoactive responses to the endothelium-dependent dilator ACh were blunted in the ischemic/reperfused LAD and the nonregionally ischemic LCx in the AB group relative to the Dil group. In addition, basal endothelial function, assessed by adherence of unstimulated neutrophils to excised epicardial coronary arteries, was decreased in the AB group. However, there were no differences between groups in terms of infarct size, plasma CK activity, segmental contractile function, or hemodynamics. Hence, both formulations of cardioplegia demonstrated acute disadvantages, but these disadvantages did not translate into differences in infarction or contractile dysfunction of the revascularized area. Although we used a crystalloid solution that maintained plasma electrolytes unchanged despite the differences in hemodilution between groups, this may have attenuated the impact of profound hemodilution with the use of other crystalloid solutions in which larger changes in plasma electrolytes may be affected by oncotic pressure, fluid extravasation, edema, extracellular calcium, and calcium-dependent processes, such as contractile shortening.

Edema

In the setting of surgical revascularization of evolving infarction, tissue edema is a consequence of pathophysiological processes stimulated by both cardiopulmonary bypass and ischemia/reperfusion injury. Cardiopulmonary bypass, per se, is associated with a whole-body inflammatory response.11,12

Figure 4. Postexperimental vasorelaxation responses in non-ischemic/reperfused LCx. A, Responses to incremental concentrations of ACh. B, Responses to incremental concentrations of SNP.
one hallmark of which is increased capillary permeability and multiorgan edema induced by proinflammatory mediators released during cardiopulmonary bypass, such as TNF-α, interleukins, and complement, TNF-α, complement, and other cytokines and chemokines are also released locally by ischemic/reperfused myocardium, thereby compounding the increase in capillary permeability and edema. The lungs, kidneys, and gut are particularly vulnerable to the capillary fragility that accompanies cardiopulmonary bypass. The degree of fluid extravasation into the tissue parenchyma is dependent in part on the oncotic properties of the blood. Accordingly, in the present study, the postexperimental tissue water content was significantly greater in the duodenum and kidney of the Dil group than the AB group; this difference was most likely due to hemodilution and the decreased oncotic properties of the blood cardioplegia and the systemic blood. In all other tissue types harvested, there was only a tendency for water content to be greater in the Dil group. The greatest tissue water content was observed in the myocardial AAR (>4% above normal). Although we expected that the myocardial AAR would sustain a greater degree of edema than the nonischemic myocardium because of the effects of normothermic ischemia and reperfusion on vascular permeability, this was not observed. There was only a tendency for greater edema in both the AAR and the nonischemic myocardium in the Dil group relative to the AB group. It is not clear from the present study whether the edema was of biological significance, ie, sufficient to affect tissue function or blood flow, or what would be the time course of resolution of the edema.

**Endothelial Injury**

Impaired endothelial function is a consequence of ischemia/reperfusion injury in both surgical and nonsurgical reperfusion settings. This endothelial dysfunction is expressed as an attenuated release (both tonic and agonist-stimulated) of the anti-neutrophil autacoid, NO. The physiological consequence of this reduced NO release is greater adherence of neutrophils to postexperimental coronary arteries and reduced vasorelaxation responses to the NO synthase stimulator ACh. Nakanishi et al reported that endothelial function after normothermic global ischemia in the absence of reperfusion was comparable to that in control vessels but was impaired after reperfusion with unmodified blood. In the present study, endothelial function in postischemic epicardial coronary arteries was quantified by two complementary assays: (1) changes in vasorelaxation responses to the endothelium-dependent agonist stimulus of NO, ACh, and (2) changes in basal function by using the adherence of unstimulated neutrophils to the endothelial surface. In the present study, both assays of postischemic coronary artery endothelial function demonstrated a modest but significantly greater impairment in function in the AB group compared with the Dil group. The greater endothelial dysfunction in the AB group may be related to the greater number of neutrophils present in the cardioplegic solution or the systemic blood. The interaction between neutrophils and postischemic endothelium causes endothelial injury, which is manifested as both obtunded vasorelaxation responses to ACh and increased adherence of unstimulated neutrophils to coronary artery endothelium. The potential consequence of this endothelial dysfunction in the AB group is a predisposition to thrombosis or greater infarction. Recent evidence has shown that neutrophil-endothelial interactions and the resulting endothelial injury influence the pathogenesis of infarction. However, in the present study, infarct size was similar between the 2 groups (discussed below), and thrombosis was not tested because of the acute nature of the study.

**Infarct Size**

The pathogenesis of myocardial infarction is largely mediated by the actions of neutrophils, particularly the generation of superoxide anion and hydrogen peroxide (via MPO activity), by the release of proteases and proinflammatory mediators, and by other cytotoxic agents that affect cell viability after ischemia and reperfusion. From numerous studies, there is a positive correlation between the degree of neutrophil accumulation and the extent of infarction. In the present study, neutrophil accumulation in the AAR showed only a tendency to be greater in the AB group, ostensibly related to a greater population of neutrophils in the systemic blood (reflected in the greater hematocrit). However, infarct size was comparable between the 2 groups. It is likely that there were countervailing forces between the 2 groups that produced equal infarct sizes in the surgical setting. The proinfarct actions of increased neutrophil delivery in the AB group may have been balanced by the proinfarct actions of hemodilution (greater vascular leak and edema) in the Dil group, thereby resulting in equivalent infarct sizes. Further studies are required to determine these countervailing forces, particularly the opposing effects of neutrophils and hemodilution in the pathogenesis of infarction.

**Summary**

The present study determined the physiological differences in using a 4:1 dilution of blood cardioplegia compared with an all-blood (66:1) cardioplegia when given in a tepid continuous retrograde modality in myocardium that was vulnerable to ischemia/reperfusion injury. Although the Dil group (with a more dilute ratio, 4:1) was associated with a progressive reduction in hematocrit and greater edema in selected organs, the AB group was also associated with negative consequences, including endothelial dysfunction. However, infarct size was comparable with both formulations of blood cardioplegic solution, suggesting that countervailing forces influenced the pathogenesis of infarction. The long-term physiological effects of this endothelial dysfunction in the AB group are not known because of the acute nature of the present study. However, the consequences of these events may include thrombosis, continued neutrophil accumulation, infarct extension, and apoptosis. On the other hand, the greater tissue edema observed in the dilute cardioplegia formulation may be associated with later tissue damage and organ dysfunction. Although there were no large-scale acute differences evident between the 2 formulations of cardioplegic solution as used in the present study, the long-term effects must be determined in appropriate models.
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References

All-Blood (Miniplegia) Versus Dilute Cardioplegia in Experimental Surgical Revascularization of Evolving Infarction

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