Microcirculatory Dysfunction Following Perfusion With Hyperkalemic, Hypothermic, Cardioplegic Solutions and Blood Reperfusion

Effects of Adenosine

Mark W. Keller, MD; Lisa Geddes; William Spotnitz, MD; Sanjiv Kaul, MD; and Brian R. Duling, PhD

**Background.** Cardioplegic solutions have been used to enhance myocardial preservation during cardiac surgery. The benefits derived from preventing myocardial ischemia with cardioplegic solutions may, however, be countered by tissue damage that occurs when the myocardium is reperfused with oxygenated blood. Furthermore, cardioplegia-induced endothelial dysfunction may contribute to depressed myocardial function postoperatively. The endothelium of coronary arteries and vein grafts is damaged by crystalloid cardioplegic solutions. There is less known about the effects of cardioplegic solutions on the microvasculature.

**Methods and Results.** The hypothesis that microvascular damage occurs following perfusion with hyperkalemic, crystalloid, cardioplegic solutions and blood reperfusion, leading to decreased blood flow and increased neutrophil accumulation, was tested in a model system. Intravital microscopic observations were performed during a 20-minute perfusion of the hamster cremaster muscle with cardioplegic solutions (10°C) via the femoral artery with the iliac occluded and during a subsequent 2-hour blood reperfusion period (iliac open). Arteriolar vasoconstriction (27% decrease in diameter, \(p<0.05\)) and a 25% decrease in the density of perfused capillaries \((p<0.05)\) occurred during reperfusion in hamsters receiving crystalloid cardioplegic solution \((16 \text{ meq K}^+)\) compared to control hamsters \((\text{no cardioplegic solution given})\). Neutrophils accumulated on venular endothelium in treated animals \((250\% \text{ increase, } p<0.05)\) and extravascularly \((\text{myeloperoxidase levels } 2.0\pm0.4 \text{ U/g versus } 1.3\pm0.3 \text{ U/g in control, } p<0.05)\). The addition of adenosine \((10^{-4} \text{ M})\) and albumin \((2 \text{ g/l})\) to the cardioplegic perfusate, accompanied by the administration of adenosine \((10^{-4} \text{ M})\) during reperfusion, produced arteriolar vasodilation \((34\% \text{ diameter increase, } p<0.05)\) and inhibited extravascular neutrophil accumulation \((\text{myeloperoxidase level } 1.5\pm0.2 \text{ U/g, } p>0.05 \text{ versus control})\). Capillary perfusion, however, was still significantly diminished \((28\% \text{ decrease, } p<0.05)\).

**Conclusions.** We conclude that injury manifest by decreased microvascular blood flow and increased neutrophil accumulation in tissues occurs after perfusion with hypothermic, hyperkalemic, crystalloid cardioplegic solutions and blood reperfusion. Adenosine seems to partially attenuate this injury by dilating arterioles and decreasing extravascular neutrophil accumulation. (*Circulation* 1991;84:2485–2494)

Cardioplegic solutions have been used to enhance myocardial preservation during cardiac surgery.\(^1,2\) The benefits derived from preventing myocardial ischemia with cardioplegic solutions may, however, be countered by tissue damage, which occurs when the myocardium is reperfused with oxygenated blood.\(^1\) Furthermore, cardioplegia-induced endothelial dysfunction may contribute to depressed myocardial function after surgery.\(^3-6\) The endothelium of coronary arteries and

---

From the Departments of Medicine, Surgery, and Physiology, University of Virginia Health Sciences Center, The University of Virginia, Charlottesville, Va.

M.W.K. is recipient of a Clinical Investigator Award (1K08 HL-02419-01A1) from the National Institutes of Health (NIH), grant-in-aid VA-90-G-40 from the American Heart Association, Virginia Affiliate, and a Biomedical Research Support Grant (Institutional Funding 2-S07RR-05431-28). B.R.D. is a Merit Awardee of the NIH and is supported by NIH grant HL-12792.

Address for correspondence: Mark W. Keller, MD, Department of Medicine, Box 158, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Received January 9, 1991; revision accepted July 16, 1991.
vein grafts is damaged by crystalloid cardioplegic solutions.3–5,7,8 There is less known about the effects of cardioplegic solutions on the microvasculature.

We suspected microvascular damage might be occurring during cardioplegic infusion and blood reperfusion in the canine heart.9 When using injections of sonicated albumin microbubbles to trace blood flow during intraoperative contrast echocardiography, we noted an increase in microbubble washout time during cardioplegic infusion compared to blood perfusion.9–12 This observation led us to hypothesize that endothelial changes were occurring that might cause microbubble adhesion.10 We also hypothesized that leukocyte adhesion might be similarly increased during reperfusion, resulting in postoperative leukocyte accumulation and, ultimately, decreased tissue perfusion.

To test these hypotheses, we developed an intravital microscopic preparation for the direct microscopic observation of all classes of microvessels before, during, and after infusion of cardioplegic solution. The cremaster muscle was selected because it is well suited for intravital microscopic observations of events in the microcirculation, which cannot be studied in the myocardium in vivo. We found evidence that the microvasculature was damaged after cardioplegic solution infusion and blood reperfusion and that adenosine may, in part, ameliorate the damage.

Methods

General Experimental Design

The experiment was performed in two phases. Experimental data consisted primarily of intravital microscopic observations in phase 1. Tissue neutrophil accumulation in the cremaster was assessed with a myeloperoxidase assay in phase 2, but intravital observations that would have interfered with the assay (e.g., fluorescent labeling of plasma or neutrophils) were avoided.

Phase 1. Four groups of five golden hamsters were studied (Table 1). The experiments were performed by intermixing the four groups throughout the course of the study. Intravital microscopy was used to assess arteriolar tone, capillary density, intravascular leukocyte behavior, and microbubble rheology. One group served as a control in which baseline and 2-hour observations were made without infusing cardioplegic solution. A second group received standard crystalloid cardioplegic solution infused at 5–10°C (referred to as the 10°C group). A third group received standard crystalloid cardioplegic solution at a temperature of 15–20°C (15°C group). In a fourth group, adenosine (10–4 M) and albumin (2 g%) were added to the cardioplegic solution that was infused at 15–20°C (adenosine group), and adenosine (10–4 M) was added to the superfusion solution during the 2-hour reperfusion period.

Phase 2. Neutrophil accumulation in the cremaster muscle (total of extravascular and intravascular) was assessed using a myeloperoxidase assay. Intravital microscopy was limited to avoid interference with the assay by fluorescent labels. Three groups of five hamsters were studied: a control group, a 10°C group, and an adenosine group, as defined above.

Animal Preparation

Thirty-five male golden hamsters (body wt, 119±10 g) were anesthetized with pentobarbital sodium (70 mg/kg i.p.), and a tracheotomy was performed to ensure a patent airway. The right internal jugular vein was cannulated for continuous infusion (420 μl/hr) of 0.9% saline containing 1 mg/ml of pentobarbital sodium for the replacement of respiratory fluid loss and the maintenance of anesthesia. Catheters were placed in the right carotid artery to monitor aortic pressure and in the right femoral artery for the infusion of cardioplegic solutions. The abdomen was opened, and a snare was placed loosely around the right iliac artery just below the aortic bifurcation. A 22-gauge catheter was inserted through the anterior wall of the bladder to permit urinary drainage. The abdominal incision was closed.

The hamster was placed on a platform, and the right cremaster muscle was prepared for intravital microscopy.13 The cremaster was superfused at approximately 5 ml/min with a bicarbonate-buffered saline solution of the following composition (mM): NaCl 131.9, KCl 4.6, CaCl2 2.0, MgSO4 1.2, and NaHCO3. The superfusate was maintained at pH 7.4 by bubbling it with 5% CO2 in nitrogen. The cremaster muscle was maintained at 34±1°C by controlling the temperature of the superfusate solution, while esophageal temperature was maintained at 36–38°C with conducted heat.

Intravital Microscopy

Microvessels of the cremaster muscle were viewed with an intravital microscope (model ACM, Zeiss). Transilluminated images were visualized

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Temp. (°C)</th>
<th>Cardioplegic composition</th>
<th>Observations</th>
<th>n</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>34</td>
<td>None</td>
<td>Intravital microscopy</td>
<td>5</td>
<td>Myeloperoxidase assay</td>
</tr>
<tr>
<td>10°C group</td>
<td>5</td>
<td>5–10</td>
<td>Standard*</td>
<td>Intravital microscopy</td>
<td>5</td>
<td>Myeloperoxidase assay</td>
</tr>
<tr>
<td>15°C group</td>
<td>5</td>
<td>15–20</td>
<td>Standard</td>
<td>Intravital microscopy</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Adenosine group</td>
<td>5</td>
<td>15–20</td>
<td>Adenosine (10–4 M), albumin (2 g%)</td>
<td>Intravital microscopy</td>
<td>5</td>
<td>Myeloperoxidase assay</td>
</tr>
</tbody>
</table>

*Standard composition (in mM): NaCl 100, KCl 16, CaCl2 1.2, MgCl2 1.6, NaHCO3 10.
with either a silicon-intensified-target tube (model SIT-66, Dage-MTI) or a charge-coupled-device (CCD 72x, Dage-MTI) equipped with an intensifier (Gen II Sys, Dage-MTI). Epi-illumination was performed with a Ploem illuminator (Zeiss) equipped with a strobe (Strobex model 236, Chadwick Helmh) that flashed at 30 Hz. The strobe was synchronized to the video vertical frame rate of the camera. Fluorochromes were examined using the following filter combinations: fluorescein; excitation filter 480, dichroic mirror 500, barrier filter 515; acridine red; excitation filter 550, dichroic mirror 580, barrier filter 590. During transilluminations, a ×50 (Leitz, N.A. 0.6) objective was used, and a ×25 immersion objective (Fluoreszenz, Leitz, N.A. 0.6) was used during fluorescence microscopy. The video images were calibrated with a stage micrometer and were recorded on a ½-in. video recorder (Sony model VO-5800H). A character generator (model G-77, Odetics) provided the time in hundreds of seconds on the recorded image.

**Fluorescent Labels, Drugs, and Cardioplegic Preparation**

**Albumin.** A solution of 5% human albumin in normal saline with sodium bicarbonate buffer (40 ml) was labeled with dichlorotriazinyl aminofluorescein (35 mg) at pH 9 by stirring at room temperature for 1 hour.\(^\text{14}\) The pH was corrected to 7.4 with HCl. Capillary density was measured by injecting 0.2 ml of the labeled albumin at the end of the experiment in phase 1 animals.

Albumin microbubbles were produced by sonication 8 ml of the labeled albumin for 40 seconds in a 10-ml syringe.\(^\text{11}\) A ½-in. sonicator horn (model XL, Heat Systems Ultrasoundics) was used, and the horn was placed below the surface for the first 10 seconds of sonication. At this point the syringe was lowered relative to the horn so that the horn tip just broke the surface, and sonication was continued for 30 seconds. The solution was allowed to stand for 3–4 hours until the bubbles had floated to the surface. The labeled albumin was then drained out of the syringe, and the bubble layer was resuspended in 8 ml of unlabeled albumin. This washing procedure was repeated two times to remove excess label. An average batch of microbubbles has a mean diameter of approximately 5 μm.\(^\text{11}\) Injections of 0.2 ml were used to assess microbubble rheology.

**White blood cell labeling.** Leukocytes were labeled in vivo by infusing 0.75 ml of a 20-mM stock solution of acridine red intravenously just before making observations.\(^\text{15}\) The stock solution consisted of 50 mg of acridine red dissolved in 10 ml of 0.9% saline using vigorous agitation. The solution was sequentially filtered through 5-μm and 0.2-μm filters to remove undissolved acridine red.

**Cardioplegic solution.** The St. Thomas hospital solution used (Plegisol, Abbott) is of the following composition (mM): NaCl 100, KCl 16, CaCl\(_2\) 1.2, MgCl\(_2\) 1.6, and NaHCO\(_3\) 10. In 10 of the hamsters, adenosine (final concentration, 10\(^{-4}\) M) and bovine albumin (dialyzed against plegisol, 2 g% as final concentration) were added to the cardioplegic solution.

**Adenosine.** A stock solution (10\(^{-2}\) M) was infused into a side port of the superfusate tubing at a rate sufficient to provide a final concentration of 10\(^{-4}\) M in the superfusate solution as it dripped onto the cremaster. Brief infusions were used to assess arteriolar tone, and a continuous 2-hour infusion was used during reperfusion in the adenosine group.

**Myeloperoxidase Assay to Assess Neutrophil Accumulation**

The tissue samples were prepared using a method modified from that of Schierwagen et al.\(^\text{16}\) The cremaster muscle was excised and homogenized in 0.02 M phosphate buffer at pH 7.4 (2 ml/0.1 g tissue). The homogenate was centrifuged at 10,000g for 5 minutes and the supernatant discarded. The pellet was homogenized in the same volume of 0.05% HTAB solution in 0.05 M phosphate buffer at pH 6.0, followed by centrifugation at 10,000g for 5 minutes.

The myeloperoxidase activity in the supernatant was measured spectrophotometrically by a modified method of Suzuki et al.\(^\text{17}\) Up to 0.1 ml of the tissue extract was added to 0.8 ml of 0.08 M phosphate buffer at pH 5.4; 0.1 ml of 16 mM TMB (3,3',5,5'-tetramethylbenzidine) was added and mixed. To start
TABLE 2. Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats per minute)</th>
<th>Diastolic blood pressure (mm Hg)</th>
<th>Systolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>CP</td>
<td>2 Hours</td>
</tr>
<tr>
<td>Control</td>
<td>410±17</td>
<td>NA</td>
<td>450±30</td>
</tr>
<tr>
<td>10°C group</td>
<td>408±26</td>
<td>342±26*</td>
<td>420±0</td>
</tr>
<tr>
<td>15°C group</td>
<td>442±29</td>
<td>372±24</td>
<td>408±50</td>
</tr>
<tr>
<td>ADO group</td>
<td>426±74</td>
<td>366±54</td>
<td>390±60</td>
</tr>
<tr>
<td>ANOVA p</td>
<td>0.69</td>
<td>0.47</td>
<td>0.3</td>
</tr>
</tbody>
</table>

CP, cardioplegic infusion; ADO, adenosine.
*p<0.05 compared to baseline measurement.
†p<0.01 compared to other groups.

the reaction, 0.15 mM H$_2$O$_2$ (final concentration) was added. The change in OD$_{655}$ was recorded for three minutes. One unit of myeloperoxidase activity was defined as that which degraded 1 µmol of peroxide per minute at 25°C.

Protocol

The animal was placed on the stage of the microscope, and baseline heart rate and blood pressure were recorded. Resting arteriolar tone was assessed with topical adenosine. The diameter was measured during adenosine application with a Colorado video on-line image analyzer. A 0.75-ml aliquot of acridine red was infused, the epi-illuminator was turned on, and baseline images were recorded of fluorescently labeled leukocytes. The fluorescence filters were changed to those for fluorescein, and a 0.2-ml aliquot of labeled albumin bubbles were injected into the femoral artery catheter. Eight microscopic fields were observed at random for fluorescent microbubbles that adhered to the endothelium.

Once baseline measurements and observations were completed, the iliac snare was tightened to interrupt blood flow to the cremaster muscle, and cardioplegic infusion was initiated by way of the tygon tubing attached to the femoral artery cannula (Figure 1). Simultaneously, the temperature of the superfusate solution was lowered to match that of the cardioplegic solution and monitored with a thermistor.

Cardioplegic solution was infused for a total of 20 minutes. This infusion period was selected to simulate the initial time that the heart is arrested after the first infusion of cardioplegic solution during bypass surgery. Normally, cardioplegic solution is infused into the cardiac circulation for several minutes to arrest the heart, and the cardioplegic solution may remain within the vasculature until the aortic crossclamp is removed and the heart is reperfused with blood. Repeat infusions may be necessary if the operation is prolonged or if significant collateral blood flow is present. Similarly, an initial 4-minute infusion of cardioplegic solution was given in this model, after which the infusion pump was stopped, and the cremaster was observed using intravital microscopy. Blood reperfusion, at a low flow rate, was observed in the majority of the preparations (probably via collaterals), and two or three repeat infusions of cardioplegic solution were necessary to keep the microvasculature free of blood for the 20-minute period. The average volume infused over the 20 minutes was 7.0±1.0 ml.

Following the first 3 minutes of cardioplegic solution infusion, a repeat injection of labeled albumin microbubbles was introduced into a sideport of the femoral artery catheter, and eight microscopic fields were viewed during epifluorescent microscopy to assess microbubble adherence. After 20 minutes, the polystatic pump was turned off, the iliac snare was released to permit reperfusion with arterial blood (blood at aortic pressure, room air ventilation), and the superfusion temperature was returned to 34°C. Reperfusion was continued for 2 hours.

The arteriolar tone was assessed after 90 minutes of reperfusion with topical adenosine. After 2 hours of recovery, 0.75 ml of acridine red was given intravenously, and at least four venules were observed during epifluorescent microscopy to assess leukocyte rheology. Capillary density was assessed after injecting 0.2 ml of labeled albumin intravenously. Five microscopic fields free of large arterioles or venules were viewed as the microscope was focused up and down through 50 µm of tissue during video recording for off-line analysis of the density of capillaries containing labeled albumin.

Image analysis. Video recordings were reviewed in slow motion on a monitor to determine capillary density and the number of adherent microbubbles. Capillaries were designated as being perfused if they were filled with labeled albumin and had flowing red blood cells. Only capillaries that intersected a horizontal reference line that bisected the video monitor were counted. Capillaries were designated as non-perfused if they were filled with albumin, but red blood cell flow was absent. The number of adherent microbubbles was determined from eight randomly selected microscopic fields and was calculated in mm$^{-2}$. Leukocyte rheology was assessed by digitizing the video tape on a Gould disk capable of storing 3,200 sequential frames. A polyethylene sheet was then attached to the Gould monitor, and the position of the venular walls and fluorescent leukocytes were drawn in with a marking pen. The digitized images were then advanced frame by frame, and the numbers of rolling and adherent leukocytes were deter-
Changes within each group over the time course of the experiment. Data values shown represent mean ± SD, unless otherwise noted.

Results

Hemodynamics

There were no significant differences in heart rate, diastolic blood pressure, or systolic blood pressure among the control, the 15°C, or the adenosine treated groups (Table 2). In the 10°C group, systolic blood pressure was significantly higher (p < 0.01) at 2 hours compared with the other groups. No other significant differences were noted between the 10°C group and the other groups.

Within each group, there were no significant differences in heart rate, diastolic blood pressure, or systolic blood pressure throughout the course of the experiment, with the exception of the 10°C group. This group demonstrated a significant decrease in heart rate (p < 0.01) during cardioplegic infusion, with subsequent recovery to baseline rate at 2 hours. In addition, the diastolic blood pressure increased significantly (p < 0.05) in the 10°C group at 2 hours.

Arteriolar Tone

Tone was tested at baseline and after 90 minutes of reperfusion in arterioles ranging from 11 to 34 μm (22.5 ± 5.8 μm) in diameter by measuring the maximal dilation produced by a brief topical application of 10⁻⁴ M adenosine, and the diameter was expressed as a fraction of the maximal diameter. There were no differences in arteriolar tone between the four groups at baseline (Figure 2, upper panel).

mired over 330 consecutive frames for each vessel analyzed. The velocity of rolling leukocytes was determined by measuring the distance traveled over time using a special measuring program with movable cursors. The number of rolling and adherent leukocytes per millimeter squared of venular endothelium was calculated from measurements of the vessel’s radius and length.

Data analysis. Data were compiled on an IBM compatible PC, and statistical analysis was performed on a VAX 8200 minicomputer using BMDP software. One-way analysis of variance was used to compare the differences between groups. Analysis of variance for repeated measures was used to assess
After 2 hours of reperfusion, the 10°C and the 15°C groups demonstrated arteriolar vasoconstriction, with resting diameters being smaller than at baseline (Figure 2, upper panel). The adenosine-treated group was nearly maximally vasodilated because of the presence of adenosine in the superfusate, and the application of additional adenosine produced a very minor increase in diameter. In the control group there was a mild vasodilation over the 2-hour observation period.

The time course of vasoconstriction is shown in Figure 2, upper panel. The data represent the average diameters of four vessels taken from the 10°C group normalized to the diameter at the beginning of blood reperfusion. A large percentage of the vasoconstriction occurred in the first 15 minutes of reperfusion. There is a continued, progressive vasoconstriction over the first 90 minutes after reperfusion.

Capillary Perfusion

There was a decrease in the density of perfused capillaries after 2 hours of reperfusion in all three groups receiving cardioplegic infusions compared with control animals (Figure 3, p<0.01). There was no significant difference in the density of perfused capillaries among the three groups that received cardioplegic solution.

The density of nonperfused capillaries was also compared. Density was defined as the number of capillaries containing labeled-albumin in which no blood flow could be seen. Although there appeared to be a mild increase in the density of nonperfused capillaries in all three groups that received cardioplegic infusions, the increase was only significant when comparing the 15°C group to the control group.

Leukocyte Measurements

**Rolling leukocytes.** There was no significant difference in the number of leukocytes rolling along the venular endothelium among the four groups at baseline (Figure 4). The number of leukocytes rolling along the venular endothelium increased significantly in the groups that received cardioplegic solution at 10°C (p<0.01) and cardioplegic solution to which adenosine and albumin had been added (p<0.05). Although the number of rolling leukocytes appeared to increase in the control and the 15°C groups, the increases were not statistically significant. There were no significant differences in the rolling velocities of leukocytes in any of the four groups at baseline or at two hours (Table 3). There was no significant difference among the four groups in the number of leukocytes that were immobile and adherent to the venular endothelium at baseline or at two hours (Table 4).

**Myeloperoxidase assay.** In phase 2, a control group (no cardioplegic given), a 10°C group, and an adenosine group were assayed. The myeloperoxidase level was significantly higher in the 10°C group than in the control group (2.0±0.40 U/g versus 1.2±0.3 U/g, p<0.05). The group treated with adenosine was not significantly different from the control group (1.5±0.2 U/g versus 1.23±0.14 U/g, p>0.05).

**Microbubble adherence.** Few microbubbles were seen to adhere in any of the groups at baseline or in the control group at 2 hours (Figure 6). However, there was a significant increase in the number of adherent microbubbles in the 10°C group (p<0.01) and in the adenosine group (p<0.05) in which cardioplegic solution was infused. Although the number of microbubbles per mm² appeared to be higher in the 15°C group, this was not statistically significant.

Discussion

A new model was developed for the direct observation of arteriolar tone, capillary perfusion, leukocyte accumulation, and microbubble rheology during and after perfusion with cardioplegic solutions. The results indicate that short-term alterations occur at all levels of the microcirculation (arterioles, capillaries, and venules) during blood reperfusion after hyperkalemic, hypothermic cardioplegic solution perfusion. The use of cardioplegic solutions decreases intraoperative ischemia, but our results indicate that there is evidence of microvascular damage during reperfusion even when cardioplegic solutions are used. This may be caused, in part, by hypoxic injury.
that occurs during cardioplegic infusion, but reperfusion injury or direct endothelial damage produced by cardioplegic solutions are also quite likely. The addition of adenosine and albumin to cardioplegic solutions accompanied by the administration of adenosine during blood reperfusion may lessen some of the microvascular changes and result in improved myocardial function after surgery.

**Changes in Arteriolar Tone**

We found significant arteriolar vasoconstriction that was independent of temperature in the range examined. Vasoconstriction was absent in the adenosine group, probably because of the direct vasodilating effect of adenosine on the vascular smooth muscle. Arteriolar vasoconstriction will increase intravascular resistance, which will lead to decreased blood flow. The mechanism of arteriolar vasoconstriction in this experiment cannot be determined from the data, but possibilities would include dysfunction of vascular smooth muscle or the endothelium.

It is possible that vascular smooth muscle may be constricted by high concentrations of potassium remaining in the interstitium after hyperkalemic cardioplegic solution infusion. However, this mechanism is unlikely because the potassium is probably cleared rapidly from the interstitium by the superfuse solution, as well as the blood, during reperfusion. Also, vasoconstriction is not present when reperfusion begins and potassium concentrations are highest. Instead, vasoconstriction begins after several minutes of reperfusion and becomes progressively greater over 90 minutes.

Endothelial dysfunction could produce vasoconstriction by a loss of endothelial dependent relaxation. Endothelial dysfunction could be caused by the direct effects of cardioplegic solution on the endothelium or could begin during reperfusion as a manifestation of reperfusion injury. Saltanha and Hearse found alterations in vascular responses to serotonin in Langendorff perfused rat hearts after the administration of hyperkalemic cardioplegic solutions, and they concluded that this was because of endothelial damage produced by cardioplegic solution. In their experiments, serotonin (5-hydroxytryptamine), which normally produces endothelial dependent vasodilation and increases coronary flow, decreased coronary flow by vasoconstriction. The higher the potassium concentration was in the cardioplegic solution, the greater the decrease in coronary blood flow seen with subsequent serotonin infusion, leading them to speculate that the endothelial dysfunction was due to the potassium. Impaired endothelial dependent vasodilation also has been demonstrated in cardiac and cerebral microvessels after periods of ischemia and blood reperfusion and is thought to be caused by the production of free radicals that inactivate endothelial dependent relaxing factor. Although cardioplegic solutions provide protection from ischemia, it is possible that free radical production occurs during reperfusion after cardioplegic infusion with a similar end result.

It also is possible that circulating vasoconstrictor substances (e.g., norepinephrine) or locally produced vasoconstrictor substances (e.g., endothelin) are more accessible to the vascular smooth muscle after cardioplegic perfusion and/or blood reperfusion because of the breakdown of the endothelial barrier to diffusion. Such mechanisms cannot be excluded.

**Capillary Perfusion**

A significant decrease in the density of perfused capillaries was seen in all groups and was not altered by adding adenosine and albumin to the cardioplegic solution. Decreased capillary perfusion will result in a decrease in nutrient delivery to tissue and a subsequent decline in tissue function. There are several possible mechanisms by which capillary perfusion may be decreased.

Increased tone in feed arteries and arterioles may reduce capillary perfusion pressure. We found an increase in arteriolar tone after perfusion with cardioplegic solutions, and this may have lowered capillary perfusion pressure enough to produce capillary stasis. There was essentially no change in capillary perfusion in the adenosine group that demonstrated

---

**TABLE 4. Adherent Immobile Leukocytes**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>2 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,283±743</td>
<td>911±553*</td>
</tr>
<tr>
<td>10°C group</td>
<td>1,380±576</td>
<td>1,843±897*</td>
</tr>
<tr>
<td>15°C group</td>
<td>1,127±688</td>
<td>931±512*</td>
</tr>
<tr>
<td>Adenosine group</td>
<td>1,946±1,225</td>
<td>2,219±1,090*</td>
</tr>
</tbody>
</table>

Values are number mm⁻² of endothelium.

*p < 0.05, no significant difference.

**FIGURE 5. Graph of myeloperoxidase activity from three experimental groups. Myeloperoxidase level in control group was lowest, and level in the 10°C group was significantly higher. Treatment with adenosine limited neutrophil accumulation, and myeloperoxidase levels were not significantly different from those in control animals. Values are mean ± SEM.**
arteriolar vasodilation, indicating that "upstream" arteriolar flow is not the sole regulator of "downstream" capillary perfusion in this model. It is possible that increased resistance in the feed arteries further upstream from the arterioles could have contributed to decreased capillary perfusion, but this was not examined in our model.

Direct damage to the endothelial lining of the capillaries could also account for decreased capillary perfusion. Intracapillary regulation of red blood cell flow is achieved, in part, by the endothelial glycocalyx, and alterations of this surface can have a profound effect on capillary perfusion.26 Rouleaux formation was observed frequently in nonperfused capillaries after cardioplegic perfusion, indicating that capillary hematocrit was markedly increased and supporting the notion that the endothelial glycocalyx was altered by the cardioplegic solutions. High capillary hematocrits will increase intracapillary blood viscosity and resistance and could lead to capillary stasis.

Mechanical occlusion of capillaries by leukocytes during blood reperfusion could also explain decreased capillary perfusion.27,28 Leukocytes were rarely seen to be plugging capillaries, but nonperfusing capillaries were not traced along their courses outside of the initial field of view to exclude leukocyte plugging. Leukocytes may accumulate in postcapillary venules, restrict the functional diameter of the lumen, and increase the resistance in upstream capillaries.28 Such a phenomenon could have led to capillary stasis, as large numbers of leukocytes were evident in postcapillary vessels during reperfusion, but the precise magnitude of this microvascular response was not estimated.

Tissue edema produced from the use of crystalloid cardioplegic solutions can lead to vascular compression and result in capillary occlusion. The addition of albumin to the cardioplegic solution in the adenosine group did not improve capillary perfusion, which makes this mechanism less likely. However, it is possible that higher concentrations of albumin (i.e., >2 g%) are necessary to prevent edema. Other mechanisms, including endothelial cell swelling or fibrin plugging, cannot be excluded.29,30

Leukocyte-Endothelial Interactions

Infusion of cardioplegic solutions at 10°C led to significant increases in the number of intravascular leukocytes and tissue myeloperoxidase levels (measure of total intravascular and extravascular neutrophil levels). The mechanism of leukocyte accumulation is not clear, but there are several possibilities, including ischemic damage to the extravascular tissue with the release of inflammatory cytokines, primary endothelial damage resulting in the expression of leukocyte binding proteins and the direct stimulation of leukocytes by the cardioplegic solution.

It is possible that ischemia, reperfusion injury, or direct effects of the cardioplegic solution could have produced tissue damage.1 There is abundant evidence that tissue damage after ischemia and reperfusion induces leukocyte accumulation.31,32 Leukocytes have also been implicated in damage to tissues during reperfusion after cardioplegic administration.33,34 Teoh et al33 demonstrated an increase in leukocytes in the myocardium during reperfusion after cardioplegic perfusion, and Breda et al34 demonstrated an improvement in myocardial function when leukocyte-free blood was used in reperfusion instead of whole blood. When appropriately stimulated, endothelial cells express receptors that enable adhesion of leukocytes to the endothelium.35–37 However, the mechanism of leukocyte accumulation was not defined in these studies.

We attempted to modify leukocyte adhesion and emigration by adding adenosine to the cardioplegic solution and to the superfusate during reperfusion. Adenosine decreased tissue accumulation of neutrophils, a finding that has been demonstrated by others.38–40 Adenosine modifies neutrophil adhesion to endothelial monolayers and cytokine-mediated neutrophil chemotaxis through receptor mediated mechanisms,41–43 and it is possible that stimulation of the adenosine receptor also may effect diapedesis.

We also found an increase in microbubble adhesion to the endothelium during cardioplegic perfusion that is not seen during blood perfusion, which may indicate a nonspecific increase in endothelial adhesiveness. Although increased microbubble adhesion is of little direct physiological importance, it is essential to consider when measuring myocardial perfusion during open heart surgery using myocardial contrast echocardiography.9 Indexes of contrast appearance (microbubble inflow) should be used to assess perfusion, and indexes of contrast clearance (microbubble washout) should be avoided.9

Mechanism of Microvascular Injury

Microvascular changes could be caused by ischemic injury that occurs during cardioplegic infusion,
direct effects of cardioplegic solutions on the endothelium, or to damage that occurs during reperfusion. We cannot exclude the possibility that hypoxic tissue damage produced the changes seen in our model; however, hypoxic injury should have been minimized in our model as the basal metabolic rate of a non-working muscle during hypothermia is low, the period of hypoxia was relatively short, and the cardioplegic solution was equilibrated with room air and may have provided some oxygen to the tissues.

There are several possible mechanisms by which crystalloid cardioplegic solutions can damage the endothelium. The effects of low oncotic pressure on the endothelial surface are well known, and it has been shown that increasing the oncotic pressure by adding albumin to cardioplegic solutions may lessen endothelial damage. Similarly, we found a benefit in the adenosine group that contained albumin, although it is possible that the optimal concentration of albumin necessary may be higher than 2 g%. Saldanha found that the higher the potassium concentration, the more severe the endothelial dysfunction, and Griffith et al used hyperkalemic solutions to remove endothelium from coronary arteries. We used the minimal concentration of potassium (16 mM) necessary to attain rapid cardiac arrest and still found evidence of damage. All of the experimental groups underwent at least moderate tissue hypothermia, and it has been demonstrated that severe hypothermia during cardioplegia can lead to tissue damage.

It is clear that reperfusion injury can occur after cardioplegia and reperfusion. Some of the changes seen in this study may have been due to reperfusion injury. Certainly, tissue damage is much worse if cardioplegia is not used. Future efforts in the development of cardioplegic solutions may be focused on limiting endothelial damage or perhaps limiting free radical production.

Study Limitations

The major limitation of the study is the experimental model. The purpose of this study was to examine the effects of cardioplegic solution infusion followed by blood reperfusion on intravascular events. Although there are major differences in muscular and vascular architecture between the myocardium and the cremaster muscle, the structure and function of the microvasculature of the two tissues are similar. The findings of this study, therefore, may be extended to the myocardial circulation. Furthermore, vascular responses of the myocardial microvessels during ischemia and reperfusion are similar to those that we found in the cremaster, although comparative data is limited to vessels that are larger than those we examined. Unfortunately, there is no available cardiac model in which to examine all of the parameters examined in this study of microvessels in the sizes and classes that we examined.

The second major limitation is the manner in which cardioplegic solution was infused. We infused cardioplegic solution intermittently over a 20-minute time period. This was done in an effort to keep the microcirculation free of blood for an amount of time similar to the time that the heart is arrested during cardiac surgery. Intermittent infusions are used during cardiac surgery if collateral blood flow is substantial, as was the case in this model, and we believe that our model is comparable to the clinical setting.

There are several limitations to the analysis of capillary perfusion. The mechanism of capillary stasis was not examined, no assessment was made of tissue edema, and the flux of red blood cells (number flowing/unit time) was not examined. Also, the ability to detect nonperfused capillaries may be inadequate, as severely plugged capillaries may not have perfused with the labeled-albumin at any time, and thereby they could have been missed on epifluorescent microscopy.

The time course of the microvascular changes, for the most part, was not examined in this study. Capillary perfusion and leukocyte accumulation were only assessed after 2 hours of reperfusion. The ability to make more frequent observations is limited by the methodology used; the background fluorescence becomes too high to make accurate observations if repetitive injections of fluorescent labels (labeled albumin and acridine red) are made.

Reperfusion with blood was not controlled in this model. Reperfusion was made with arterial blood at aortic pressure (the cardioplegia column of Table 2). The partial pressure of oxygen was not measured routinely, but it average 97 mm Hg in the hamsters in which it was measured.

Finally, it is not possible to make a recommendation on the optimal cardioplegic solution to use based on our data, but the purpose of this study was not to define such a solution. It is clear that the use of cardioplegic solutions lessens tissue damage. The addition of adenosine and albumin to the St. Thomas solution may provide additional benefit.

Importance. Damage to the microvasculature may adversely effect organ perfusion and function. Therefore, the finding of microvascular damage after cardioplegic solution perfusion and blood reperfusion may explain a portion of the decrease in myocardial function seen after cardiac surgery. The addition of albumin and adenosine to crystalloid, hyperkalemic solutions may be beneficial. The model used in this study enabled microvascular injury to be detected by direct observation and added considerable support to pathophysiological events implied by the indirect findings of other investigators.

References

23. Lefer AM, Tsao PS, Lefer DJ, Ma XL: Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. FASEB J 1991;5:2029–2034

**Key Words**: cardioplegia • reperfusion injury • leukocytes • endothelium • microbubbles • arterioles • capillaries
Microcirculatory dysfunction following perfusion with hyperkalemic, hypothermic, cardioplegic solutions and blood reperfusion. Effects of adenosine.
M W Keller, L Geddes, W Spotnitz, S Kaul and B R Duling

Circulation. 1991;84:2485-2494
doi: 10.1161/01.CIR.84.6.2485

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/84/6/2485

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/