Hyperbaric oxygen solution infused into the anterior interventricular vein at reperfusion reduces infarct size in swine

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Submitted 1 May 2003; accepted in final form 1 May 2004

Hyperbaric oxygen has been shown to reduce ischemic myocardial injury in experimental and clinical studies (21, 25, 26, 27, 31). Initial work using hyperbaric oxygen chambers showed efficacy to salvage myocardium but have limited practical applicability. Possible mechanisms for myocardial salvage by hyperbaric oxygen solution include restoration of oxygen to mitochondria and maintenance of oxidative metabolism and effects of oxygen to inhibit neutrophil activity (4). Neutrophil activation can be quantified by measuring myeloperoxidase (MPO) activity (16, 20). In a proof of concept paper, Spears and co-workers (22, 23) described a bubbleless method for dissolving oxygen at partial pressures of 3–10 MPa (30–100 bar) into crystalloid solutions delivered at a rapid velocity through capillary tubes. This principle is based on the observation that cavitation thresholds are lowered by ejecting liquids at high velocity and high hydrostatic pressures through capillary tubes of small dimensions. The oxygenated solution (AO) can be administered to host blood at ambient pressure without enucleation and thereby delivering blood with increased oxygen content to tissues via a catheter delivery technique. A method of delivery of hyperbaric oxygen solution to the myocardial risk region (RR) via a venous route may have wider applicability than antegrade infusion through coronary arteries, and therefore the present study was designed to test the hypotheses that retro perfusion of AO into the coronary venous system can diffuse into the RR and increase tissue oxygen levels and that administration of AO via the venous route can salvage myocardium by reducing reperfusion injury associated with reduced neutrophil activation.

MATERIALS AND METHODS

This study was performed within the guidelines specified by the National Institutes of Health for the care and use of laboratory animals and with the approval of the Rhode Island Hospital Animal Care Committee. Castrated juvenile male swine weighing 35–50 kg were used.

Animal preparation and instrumentation. Animals were given an intramuscular anesthetic (1 mg/kg Telazol-xylazine). An ear vein was cannulated, and 2% intravenous pentothal sodium was given to deepen anesthesia. The animal was then intubated and placed on a ventilator, and anesthesia was maintained with inhaled isoflurane at 0.5–1.25% and nitrous oxide (2 l/min). Cutdowns were performed over the right carotid artery, right external jugular, and femoral artery. The right carotid artery was used for the balloon catheter, and the right external jugular vein was used for the anterior interventricular vein catheterization site. A femoral artery was used for arterial pressure monitoring.

REPERFUSION THERAPY for acute myocardial infarction has reduced morbidity and mortality; however, it has been recognized for many years that restoring antegrade blood flow into the coronary circulation may not uniformly improve nutrient perfusion (8). Although initially thought to be due to the ischemic insult itself, experimental evidence points to events in the early reperfusion period (1). Possible culprits include endothelial damage by oxygen free radicals, altered vascular reactivity, cell swelling, and damage due to attraction and activation of neutrophils (11, 12, 14).
and withdrawal of arterial blood for the oxygenation. An angiogram of the left anterior descending coronary artery (LAD) was obtained with emphasis on late venous drainage of the LAD territory. An 8-Fr right Judkins guide catheter with the AO infusion catheter loaded inside (Tracker-38 catheter, Cork; Cork, Ireland) was positioned via the coronary sinus in the great cardiac vein at the base of the heart. A 0.014-in. guide wire was advanced through the AO catheter and into the anterior interventricular vein (AIV), which parallels the LAD. The AO catheter was then advanced over the wire into the distal AIV, the infusion site for the AO solution. An occluder balloon catheter was positioned in the mid to distal LAD territory. Heparin (300 mg/kg) was given after cutoffs were completed and before instrumentation. The activated clotting time was maintained between 300 and 400 throughout the remainder of the procedure with monitoring every 30 min. At this time, the animal was switched to intravenous anesthetic (2.5% pentothal at 20–30 ml/h) and inspired gas of 100% fraction of inspired oxygen. Arterial blood gases were sampled every 30 min with the aim to keep the PO₂ in the range of 400–500 mmHg.

Open-chest experiments to measure myocardial O₂. Three open-chest experiments were performed to measure myocardial levels of oxygen. A limited sternotomy was performed exposing only the distal anterior wall of the left ventricle (LV). An optical sensing O₂ probe (FOXY, Ocean Optics; Dunedin, FL) was used to sample myocardial oxygen concentration. This system comprises fiber-optic oxygen sensors that use the fluorescence of a ruthenium complex in a sol-gel to measure the PO₂. A pulsed blue LED excites the ruthenium complex at the probe tip and causes it to fluoresce. Oxygen molecules quench the fluorescence signal, and the degree of quenching correlates to the level of oxygen concentration in the tissue. The probe was positioned just proximal to the AIV catheter tip, and baseline readings were taken. After baseline O₂ tissue measurements, the LAD was occluded for 60 min. Myocardial tissue O₂ was monitored baseline, during occlusion, and for 120 min of reperfusion.

Two control experiments were performed. The first control received no AIV infusion at reperfusion; the second control received autoperfusion with arterial blood into the AIV immediately at reperfusion and continued for 90 min. In the third experiment (treatment), hyperbaric oxygen was infused into the AIV immediately at the time of reperfusion and continued for 90 min. The AO system comprised a pressurized AO vessel, mixing chamber, and tubing set. Arterial blood was withdrawn from a femoral artery, hyperoxygenated via AO infusion into a mixing chamber, and then delivered to the infusion site via the AO catheter located in the AIV. The oxygen level of blood delivered into the AIV was calculated to be in the range of 1,000–1,400 mmHg, infused at a rate of 75 ml/min.

Microsphere injections for blood flow measurements were made at the end of instrumentation, at 60 min of LAD occlusion just before reperfusion, and at 2 h postreperfusion. Transthoracic echocardiograms were performed to evaluate global and regional LV function at the end of instrumentation and at 50 min of LAD occlusion just before reperfusion at two to three times during reperfusion. An Agilent 5500 Sonos platform with a phased array 2–4 MHz probe was used. Animals were imaged in four standard imaging planes: parasternal long axis, parasternal short axis, apical four-chamber, and apical two-chamber views.

Two control experiments were performed. The control experiment without anterior interventricular vein (AIV) infusion during reperfusion (PO₂-con1); control experiment with arterial blood autoperfusion into the AIV at reperfusion (PO₂-AO); treated experiment with oxygenated solution (AO) infusion into the AIV at reperfusion (PO₂ AO). The table shows mean transmural blood flow (MBF) measurements in the risk region baseline and at the end of occlusion and reperfusion periods.
Euthanization and pathology. At the completion of the last microsphere blood flow measurement and echocardiogram, the chest was opened and the coronary artery was ligated at the balloon position. Fluorescein dye was injected into the left atrium to stain the heart excluding myocardium perfused distal to the ligation (RR). The animal was then given a large dose (40 ml) of pentothal by intravenous push, immediately followed by a lethal dose (20 ml) of supersaturated KCl through the left atrial catheter. The heart was removed, washed, and sliced into 1-cm slices. The RR was demarcated from the fluoroscin staining in black light. Transmural samples averaging 0.15 g were taken from the RR, border zone, and normal myocardium fixed in 10% neutral buffered formalin overnight. Video images of the stained slices of the heart were obtained using a computer graphics workstation (Image Pro Plus, Media Cybernetics; Carlsbad, CA), color regions for infarct and noninfarct areas were defined. The infarct area, RR, and total myocardial area were planimetered for each slice, and the infarct size was calculated as percentage of the RR and as percentage of myocardium.

Regional myocardial blood flow values were measured using the methods described by Hale et al. (9). Whole weight tissue samples of <3 g were hydrolyzed in 2 N NaOH solution overnight and then Tissue Blood Digest Reagent II. Samples were centrifuged, and the pellets were washed twice with Microsphere Counting Reagent. The numbers of colored microspheres in the final tissue and reference blood preparations were counted manually using a Fuchs-Rosenthal hemacytometer counting slide. Either a total of 400 microspheres of each color was counted or counting was done until the sample was gone. Blood flow values for risk region and normal zone for each experiment are averaged over the myocardial slices comprising the area.

Statistical analysis. All group values are expressed as means ± SD. Between-group and intragroup differences were analyzed using ANOVA.

RESULTS

Open-chest experiment with O2 probe. The results of the three open-chest experiments are shown in Fig. 1. In all three experiments, P O2 fell with occlusion into the range of 20–40 mmHg. At reperfusion, the tissue P O2 increased to the baseline level or higher briefly and then fell to baseline levels until about 60 min when in the two control experiments the P O2 values fell into the occlusion range, whereas in the experiment with AO treatment at reperfusion P O2 gradually rose to plateau in the 80s until the experiment was terminated at 120 min. Average blood flow values in the risk region also diverged between the two control experiments and the treated experiment later in reperfusion. In the control experiment, the blood flow value dropped into the occlusion range, whereas in the AO-treated experiment it increased to a value higher than control.

Hemodynamic and myocardial blood flow data. There were no statistically significant differences in heart rate, mean arterial pressure, or mean coronary pressure among the three groups (control, 30 min treated, 0 min treated) for baseline, occlusion, and reperfusion (Table 1). The mean arterial P O2 values at baseline for the three groups were 509 ± 125, 469 ± 110, and 485 ± 125 mmHg, respectively. Between-group and intragroup differences were analyzed using ANOVA.
36, and 424 ± 106 mmHg (P = 0.89). The mean arterial Po2 values during occlusion for the three groups were 511 ± 104, 444 ± 91, and 480 ± 29 mmHg. For reperfusion, the Po2 values for the three groups were 497 ± 93, 463 ± 18, and 485 ± 71 mmHg (P = 0.94).

Endocardial and transmural blood flow values and values for baseline, occlusion, and reperfusion states for the three groups are depicted in Table 2. There were no significant differences in transmural or endocardial blood flow values for baseline or occlusion among the three groups, but for reperfusion endocardial blood flow was significantly higher in the group treated with AO at reperfusion (P = 0.01). Transmural blood flow during reperfusion in the risk region was borderline higher in the treated animals (P = 0.08). Although mean values for RR blood flow were only modestly reduced in control and 30-min reperfused experiments, there was a heterogeneity of individual blood flow values measurements within each group. The means of the lowest reperfusion RR values for the five experiments in each group were 0.23 ± 0.14 ml·g⁻¹·min⁻¹ for controls, 0.19 ± 0.04 ml·g⁻¹·min⁻¹ for the 30-min treated group, and 0.40 ± 0.04 ml·g⁻¹·min⁻¹ for the AO-treated group, significantly higher than for the other two groups (P = 0.006).

**Infarct size.** There were no differences in the RRs among the three groups. The mean RR area as a percentage of the LV for the controls was 28 ± 4%, for the 30-min-treated group was 34 ± 7%, and for the 0-min-treated group was 30 ± 6%. Infarct sizes as percentages of the LV and as percentages of the RR are shown in Fig. 2. There was no statistically significant difference between infarct size as either a percentage of the LV or percentage of the RR between the control group and the group with AO begun at 30 min into reperfusion, whereas the infarct size as a percentage of the LV and as a percentage of RR in the 0-min-treated experiments were significantly different from the other two groups (P < 0.01). Representative TTC-stained slices are shown in Fig. 3.

**MPO levels.** MPO levels are shown in Table 3. Mean values for epicardial, midmyocardial, and endocardial layers were significantly lower in experiments treated at 0 min compared with controls and experiments treated at 30 min.

**LV function.** Results for serial global and regional LV function from baseline through 60 min of reperfusion for the three groups are shown in Table 4. There were no significant differences among groups for global LV function and regional wall thickening in the RR or remote regions for baseline, occlusion, or at 90 min of reperfusion. Not all animals were...
impaired coronary artery may be patent, the distal coronary bed may have reduced mortality in all patients. Although the epicardial coronary collateral zone alone does not optimally salvage myocardium and myocardial infarction patients. However, reperfusing the infarct zone immediately at reperfusion and up to 30 min of reperfusion but found zones within the ischemic zone that showed a fall in blood flow later in reperfusion. The tissue in these zones was characterized by absent collateral flow during ischemia, contraction band necrosis, and intracapillary erythrocyte stasis and neutrophil accumulation.

Experimental and clinical studies using different imaging modalities have documented reperfusion injury in patients. Blood flow to the infarct beds perfused by open coronary vessels (thrombolysis in myocardial infarction grade 3 flow) was semiquantitatively scored based on the presence and the rate of clearance of the myocardial blush of contrast (8). In 762 patients enrolled in the thrombolysis in myocardial infarction 10B trial, mortality rates correlated with the infarct bed perfusion. Impaired myocardial perfusion characterized by absence of myocardial contrast blush or absence of clearance of the blush from the first injection was associated with highest mortality. Noninvasive imaging technologies that can serially monitor blood flow to the myocardium including PET imaging and MRI have documented progressive reductions in myocardial blood flow during reperfusion. In canine models, myocardial blood flow measured by rubidium-82 decreased progressively from 2 to 4 h after reperfusion, and serial gadolinium-diethylenetriaminepentaacate MRI showed progressive increase in hypoenhanced region in the infarct zone over 48 h after reperfusion (10, 19). MRI hypoenhancement correlated with an increased risk of cardiovascular events (32).

Proposed mechanisms for this reperfusion injury after prolonged ischemia have included local release of oxygen free radicals, hemorrhage, and the inflammatory response caused by neutrophils (11, 12). Neutrophils are a major component of the inflammatory response of postischemic injury and are prominent in zones of no reflow (6, 7, 18). Severe ischemia has been shown to induce the expression of adhesion molecules that attract neutrophils to adhere to the endothelial surface of capillaries and migrate into the myocardium, where degranulation with a release of proteases and other proinflammatory mediators leads to cell death (15, 24). Quantifying neutrophil infiltration of the myocardium by histomorphometry is laborious and subject to limitations. Methods were developed to measure neutrophils in tissue by measuring the activity of the neutrophil-specific enzyme MPO (16). This assay has been shown to correlate with neutrophil cell number in tissue and initial recovery in blood flow immediately at reperfusion and up to 30 min of reperfusion but found zones within the ischemic zone that showed a fall in blood flow later in reperfusion. The tissue in these zones was characterized by absent collateral flow during ischemia, contraction band necrosis, and intracapillary erythrocyte stasis and neutrophil accumulation.

Data from the experiments described above support the premise that increasing myocardial levels of oxygen at the time of reperfusion reduces reperfusion injury but that delaying treatment by 30 min does not reduce injury. In the open-chest experiments, myocardial oxygen levels and blood flow values were higher than control up to 2 h into reperfusion. Endocardial blood flow in the RR during reperfusion was significantly higher and infarct sizes were significantly smaller in animals treated immediately at reperfusion. The higher myocardial blood flow and tissue O2 and lower MPO levels during reperfusion support the premise that microvascular integrity was better maintained due to the effect of O2 to reduce the inflammatory response. Although infarct size was smaller, the LV ejection fraction at 60 min of reperfusion was not higher in the AO 0-min-treated group compared with the control or AO 30-min-treated groups, probably due to insufficient time for recovery from contractile dysfunction.

Thrombolytic therapy and/or angioplasty have reduced infarct size and consequent morbidity and mortality for acute myocardial infarction patients. However, reperfusing the infarct zone alone does not optimally salvage myocardium and reduce mortality in all patients. Although the epicardial coronary artery may be patent, the distal coronary bed may have impaired flow. Ambrosio and co-workers (1) documented the initial recovery in blood flow immediately at reperfusion and up to 30 min of reperfusion but found zones within the ischemic zone that showed a fall in blood flow later in reperfusion. The tissue in these zones was characterized by absent collateral flow during ischemia, contraction band necrosis, and intracapillary erythrocyte stasis and neutrophil accumulation.

**Table 3. Myeloperoxidase**

<table>
<thead>
<tr>
<th></th>
<th>Epi RR</th>
<th>Epi Ratio</th>
<th>Mid RR</th>
<th>Mid Ratio</th>
<th>Endo RR</th>
<th>Endo Ratio</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
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<td></td>
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<tr>
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<td>1.93</td>
<td>6.01</td>
<td>2.80</td>
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<td>1.58</td>
<td>5.00</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Mean</td>
<td>2.58</td>
<td>21.44</td>
<td>2.17</td>
<td>17.76</td>
<td>2.03</td>
<td>13.58</td>
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<tr>
<td>SD</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>0.32</td>
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<td>0.36</td>
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<td>SD</td>
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<td>2.08</td>
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<td>&lt;0.001</td>
<td>0.03</td>
<td>0.008</td>
<td>&lt;0.001</td>
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Values are in units of myeloperoxidase. Epi, epicardial; mid, midmyocardial; Endo, endocardial; RR, risk region.

**DISCUSSION**

The results of this investigation support the premise that increasing myocardial levels of oxygen at the time of reperfusion reduces reperfusion injury but that delaying treatment by 30 min does not reduce injury. In the open-chest experiments, myocardial oxygen levels and blood flow values were higher than control up to 2 h into reperfusion. Endocardial blood flow in the RR during reperfusion was significantly higher and infarct sizes were significantly smaller in animals treated immediately at reperfusion. The higher myocardial blood flow and tissue O2 and lower MPO levels during reperfusion support the premise that microvascular integrity was better maintained due to the effect of O2 to reduce the inflammatory response. Although infarct size was smaller, the LV ejection fraction at 60 min of reperfusion was not higher in the AO 0-min-treated group compared with the control or AO 30-min-treated groups, probably due to insufficient time for recovery from contractile dysfunction.

Thrombolytic therapy and/or angioplasty have reduced infarct size and consequent morbidity and mortality for acute myocardial infarction patients. However, reperfusing the infarct zone alone does not optimally salvage myocardium and reduce mortality in all patients. Although the epicardial coronary artery may be patent, the distal coronary bed may have impaired flow. Ambrosio and co-workers (1) documented the initial recovery in blood flow immediately at reperfusion and up to 30 min of reperfusion but found zones within the ischemic zone that showed a fall in blood flow later in reperfusion. The tissue in these zones was characterized by absent collateral flow during ischemia, contraction band necrosis, and intracapillary erythrocyte stasis and neutrophil accumulation.

**Table 4. Left ventricular function**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>Occl</th>
<th></th>
<th>Rep</th>
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<tr>
<td></td>
<td>Fiber shortening, %</td>
<td>LVEF, %</td>
<td>%WT RR</td>
<td>%WT remote</td>
<td>Fiber shortening, %</td>
<td>LVEF, %</td>
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<tr>
<td><strong>Controls</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>61</td>
<td>37</td>
<td>34</td>
<td>18</td>
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<tr>
<td>SD</td>
<td>2.6</td>
<td>1.2</td>
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<td>6.8</td>
<td>3.0</td>
<td>6.6</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>30</td>
<td>58</td>
<td>35</td>
<td>30</td>
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<td>36</td>
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<tr>
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<td>9.8</td>
<td>1.7</td>
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<td>5.9</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<tr>
<td><strong>P value</strong></td>
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<td>0.36</td>
<td>0.77</td>
<td>0.29</td>
<td>0.5</td>
<td>0.78</td>
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LVEF, left ventricular ejection fraction; WT, wall thickening.
improved methods developed to reduce enzyme inhibition by the tissue (20).

The concept of reducing infarct size by delivering hyperbaric levels of oxygen to the heart has been pursued for many years. The initial approach used hyperbaric chambers (21, 25, 26, 27, 31). In most of the animal experiments, hyperbaric chamber treatment was performed during the ischemic phase and showed a reduction in infarct size. However, in one canine study (31), animals underwent coronary occlusion for 90 min and were then treated either with hyperbaric O2, reperfusion, or reperfusion plus hyperbaric O2 delivered at the time of reperfusion. The last group showed improved myocardial salvage over reperfusion alone (31). Hyperbaric chamber treatment, while showing efficacy of therapy to reduce infarct size, is logistically cumbersome and requires a hyperbaric unit. Other approaches have been developed to locally administer oxygen to ischemic tissue. One approach was the use of blood-free reperfusion with oxygenated perfluorocarbon (13). This approach was shown to reduce injury by protecting endothelial cells. A method to deliver hyperbaric oxygen in solution was developed by Spears and co-workers (23). They developed a method for dissolving oxygen at a partial pressure of 3 to 10 MPa (30–100 bar) in physiological crystalloid solutions. In this technique, the oxygenated solution is delivered at high pressures through microdiameter silica tubes and is not based on membrane oxygenator technology. After the AO solution passes through the capillary tube, it is mixed with the subject’s arterial blood. These investigators have demonstrated correction of regional hypoxemia using AO infusion in different animal models (22, 23). They have also demonstrated a reduction in infarct size when AO solution is infused antegrade into the coronary arteries of swine (22). This approach requires coronary catheterization, whereas the coronary venous approach requires a right heart catheterization.

The possible mechanisms for myocardial salvage by hyperbaric O2 solution include restoration of oxygen to mitochondria and maintenance of oxidative metabolism and effects of oxygen to inhibit neutrophil activity. Neutrophils are attracted to sites of inflammation by increased avidity of cell surface CD11/18 heterodimeric protein complexes to bind to the ligand ICAM-1 on endothelial cells (15, 24). Hyperbaric oxygen reduces CD18-dependent neutrophil adhesion in vitro (28, 30). Studies have investigated the mechanism for these observations. Although not conclusive, data support the premise that hyperbaric oxygen may inhibit membrane guanylate cyclase through nitric oxide (2, 5). Hyperbaric oxygen has been shown to induce endothelial nitric oxide synthases mRNA and protein production (3, 17). Nitric oxide may suppress guanylate cyclase, which interferes with CD18 function (2). Another possible mechanism for the beneficial effect of hyperbaric oxygen to reduce reperfusion injury is the observation of an apparently paradoxical reduction in oxygen free radicals through effects of high levels of oxygen to antagonize lipid peroxidation through oxygen-mediated termination reactions (29).

The present study extends the experience using hyperbaric oxygen as a therapeutic approach to reduce reperfusion injury. A coronary venous approach is potentially more applicable and safer than a coronary arterial approach. Further work is needed to explore whether infusion into the great cardiac vein or coronary sinus can also effectively perfuse the RR to facilitate coronary venous AO therapy in patients.

**Limitations.** Although tissue Po2 was measured in one experiment in each group, these data cannot be extrapolated to all the closed-chest experiments. The catheter delivering the hyperbaric oxygen solution was not occlusive. The ability to backperfuse the RR is probably due to the relatively high flow of the delivery system, but there is no proof that this is the correct explanation. There was a high degree of variance for the values of infarct size as a percentage of the heart and as a percentage of the RR. In the balloon occlusion LAD infarct model used in these experiments, it is technically very difficult to create infarcts of uniform size among animals due to variability in number and location of diagonal branches. The presumptive conclusion that high oxygen reduces the inflammatory response is based only on data showing fewer neutrophils adherent or accumulated somewhere in the area at risk without confirmatory evidence to show an attenuation of the inflammatory response in the RR of animals treated with AO solution. The study was a nonsurvival protocol, and LV function was monitored for 60 min after reperfusion was established. The study would have been strengthened if the animals were allowed to survive for several days to extend the time for monitoring for any recovery of LV function.

**ACKNOWLEDGMENTS**

We thank T. Donahay for help with preparing the manuscript.

**GRANTS**

This study was done through a grant from TherOx.

**DISCLOSURES**

P. Zalesky and J. Creech own stock and hold patents (one pending) with TherOx.

**REFERENCES**


