Hyperbaric Oxygen Limits Infarct Size in Ischemic Rabbit Myocardium In Vivo

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Background. We explored the ability of increased oxygen pressure to modify necrosis in an open-chest rabbit model of myocardial ischemia and reperfusion.

Methods and Results. A branch of the left coronary artery was occluded for 30 minutes followed by 3 hours of reperfusion. Infarction was measured by triphenyl tetrazolium staining and expressed as a percentage of the ischemic zone. Untreated rabbits were ventilated with 100% oxygen at 1 atm absolute. Treatment animals were exposed to hyperbaric oxygen at 2.5 atm absolute. The 1.0-atm control hearts developed 41.5±4.6% infarction of the ischemic zone. Animals exposed to hyperbaric oxygen during ischemia only, reperfusion only, or ischemia and reperfusion had significantly smaller infarcts with respect to control animals (16.2±2.9%, 14.5±3.7%, and 9.8±2.7%, respectively; P ≤ .01), indicating that they had been protected by the procedure. When hyperbaric oxygen was begun 30 minutes after the onset of reperfusion, no protection was seen (35.8±3.8%).

Conclusions. We conclude that hyperbaric oxygen limits infarct size in the reperfused rabbit heart and that the effect can be achieved when hyperbaric oxygen is begun at reperfusion. (Circulation. 1993;88[part 1]:1931-1936.)

KEY WORDS • hyperbaric oxygen • myocardial infarction • free radicals • cardioprotection • reperfusion

Myocardial infarction is a major complication of coronary artery disease. Myocardial cells do not die simultaneously in the ischemic ventricle but rather can be seen to die as a “wave front” advancing from the subendocardium to the epicardium as the duration of the ischemic period is increased. The wave front is a result of a transmural gradient in residual perfusion and innate differences in the vulnerability of individual cells to ischemia. Since oxygen is thought to be the critical metabolite in ischemia, it has been reasoned that augmenting the oxygen delivery to the ischemic heart should serve to limit or at least delay necrosis in an evolving myocardial infarction. Indeed, oxygen therapy at 1 atm has long been an adjunctive therapy for ischemic heart disease.

Several studies have attempted to augment oxygen delivery to ischemic myocardium with hyperbaric oxygen in animal models of permanent coronary artery occlusion and acute myocardial infarction, but the experimental and clinical results were mixed. Consequently, hyperbaric oxygen did not gain widespread approval or usage. Unfortunately, these early trials were conducted before the advent of thrombolytic therapy, which allows rapid restoration of perfusion to the ischemic myocardium. It is not surprising that hyperbaric oxygen would be ineffective with permanent coronary artery occlusion. Improvements in myocardial oxygenation obtained with hyperbaric oxygen exposure might temporarily interrupt the ischemic process, but ischemic degeneration would be expected to resume after the subject is removed from the chamber. If hyperbaric oxygen were used as an adjunct to thrombolysis, however, it might be able to delay necrosis until reperfusion could be established.

Whereas augmented oxygen delivery during ischemia should delay cell death, elevated oxygen tension during reperfusion may actually promote necrosis. Over the past decade, data have accumulated suggesting that reperfusion contributes to infarction via metabolically produced partially reduced reactive oxygen species (PRROS). If the reperfusion injury theory is true, then hyperbaric oxygen therapy obviously would be contraindicated in acute myocardial infarction patients at the time of reperfusion, since PRROS production should be greatly accelerated by high partial pressures of oxygen (PO₂) through mass action. However, after more than a decade of research on PRROS and the ischemic heart, there is still no consensus as to whether oxygen radicals contribute to the infarction process.

In light of the above analyses, it is not clear whether hyperbaric oxygen would limit or increase infarct size in an ischemia-reperfusion model; therefore, we decided to test the effect of hyperbaric oxygen on infarct size in a model of transient regional ischemia in the rabbit heart. Because hyperbaric oxygen could have a different effect during ischemia than at reperfusion,
the experiment was designed so that exposure to hyperbaric oxygen could be limited to the ischemic period only, the reperfusion period only, or to both periods.

Methods

Surgical Preparation of Animals

New Zealand White rabbits of either sex, weighing between 1.34 and 2.29 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) administered via a marginal ear vein. Anesthesia was supplemented in 3-mg boluses as needed throughout the study. Anesthesia was indicated whenever respiratory movements were observed (about every 20 minutes). The neck was opened with a ventral midline incision, and a tracheotomy was performed. Once the surgery was completed, all animals were studied in a Plexiglas hyperbaric chamber (14-in. inside diameter and 48 in. long). The chamber was pressurized with room air, and the rabbits were ventilated with 100% oxygen via a pressure-driven respirator (MD Industries, Mobile, Ala). The ventilation rate was set between 30 and 35 breaths per minute, with a tidal volume of approximately 15 mL. Fig 1 shows the ventilation scheme. Briefly, pressure within the chamber served as the reference for the regulator delivering 100% oxygen to the input of the respirator. Thus, the respirator was always supplied with oxygen at chamber pressure plus 100 mm Hg. The rabbit exhaled passively into the chamber. The ventilation rate was adjusted to keep the blood pH within the physiological range. Catheters flushed with heparinized saline (10 U/mL) were placed in the left carotid artery and the right jugular vein to monitor blood pressure and administer additional anesthetic, respectively. These catheters were connected to ports in the chamber door so that they could be accessed even when the chamber was pressurized. The chamber was always pressurized or decompressed gradually over a 4- to 5-minute period. A left thoracotomy was performed in the fourth intercostal space, and the pericardium was opened to expose the heart. A 2.0 silk with an RB taper needle was passed around a branch of the left coronary artery, and the ends of the silk were tied to form a loop that was used as the snare. The rabbit was then placed into the hyperbaric chamber, where the snare line covered with vinyl tubing was attached to the secured loop. The coronary branch was occluded by pulling the snare through the tubing and clamping the tubing with a mosquito hemostat. Temperature in the chamber was monitored with a thermometer and did not appreciably change with compression or decompression. Myocardial ischemia was confirmed by regional cyanosis and a drop in blood pressure. Reperfusion was created by releasing the snare and was confirmed by the visible observation of hyperemia over the surface.

Fig 1. Diagram shows how rabbits were ventilated in the hyperbaric oxygen chamber. The ventilator requires a pressure of 100 mm Hg above tracheal pressure. In the upper right, a regulator has been modified so that the reference port is connected to the chamber. Thus, once set, the regulator delivers gas at chamber pressure plus 100 mm Hg. This ensures that tidal volume will remain constant regardless of the chamber pressure. Note that the exhaled gas is released directly into the chamber, which is continuously flushed with compressed air.
Measurement of Infarct and Risk Volume

At the end of each experiment, the heart was quickly removed and mounted on a Langendorff apparatus and flushed with room temperature saline for 60 seconds. The silk suture under the coronary branch was tied securely to occlude the artery, and a 0.5% suspension of zinc cadmium sulfide fluorescent particles (1- to 10-μm diameter; Duke Scientific Corp, Palo Alto, Calif) was infused into the perfusate to differentiate the risk zone as the tissue with no fluorescence. This differentiation was confirmed by ultraviolet light before removing the heart from the Langendorff apparatus. Upon removal, the heart was weighed and frozen. When frozen, the heart was cut into 2-mm-thick transverse slices. After thawing, the slices were stained by incubation for 20 minutes in 1% triphenyl tetrazolium chloride in pH 7.4 sodium phosphate buffer. Tetrazolium reacts with reduced nicotinamide adenine dinucleotide (NADH) in the presence of dehydrogenase enzymes and causes all tissue still having the enzymes and cofactors to stain a deep red color. Infarcted areas lose enzymes and cofactors within 2 to 3 hours and therefore do not show signs of pigment formation. The slices were then soaked in 10% formalin to enhance the contrast of the stain. Upon removal from the formalin, the slices were pressed between two glass plates to a uniform 2-mm thickness. The infarcted and risk zone regions were traced through the glass. The infarct and risk zone areas were determined by planimetry of the tracings. The volumes of infarcted myocardium and myocardium at risk were calculated by multiplying the planimetered areas by the slice thickness.

Experiment Protocols

This experiment involved 65 animals divided into 6 groups. All treatment animals were subjected to a 30-minute coronary occlusion followed by 180 minutes of reperfusion. Except for the pressure control group, all groups were ventilated with 100% oxygen. Group 1, the ambient pressure control group, underwent the 210-minute procedure in the hyperbaric chamber but breathing 100% oxygen at 1 atm absolute. These rabbits could be considered as comparable to patients receiving conventional oxygen therapy. Group 2, the hyperbaric oxygen–ischemia group, experienced the 30-minute occlusion at 2.5 atm absolute pressure. The chamber was decompressed 5 minutes before reperfusion so that reperfusion occurred at 1 atm absolute. Group 3, the hyperbaric oxygen–reperfusion group, experienced the 30-minute occlusion at 1 atm absolute pressure. The pressure was then increased to 2.5 atm absolute 5 minutes before the release of the snare, and the heart was reperfused at 2.5 atm absolute for 180 minutes. Group 4, the hyperbaric oxygen–ischemia plus reperfusion group, experienced the entire 210-minute ischemia and reperfusion procedure at 2.5 atm absolute. Group 5, the hyperbaric oxygen–late reperfusion group, experienced the occlusion and 30 minutes of reperfusion at ambient pressure. Thirty minutes into the reperfusion, the pressure was increased to 2.5 atm absolute for the remaining 150 minutes of the experiment. A final group, group 6, served as a pressure control group; the animals were ventilated with 40% oxygen/60% nitrogen at 2.5 atm absolute pressure for the entire 210-minute proce-
percent of the risk zone infarcted in the pressure control group was not different from that seen in the ambient pressure control group, indicating that high pressures per se were not protective. Infarct sizes in the hyperbaric oxygen–late reperfusion group were also not different from those in the ambient pressure control group, indicating that hyperbaric oxygen treatment can be protective in the reperfusion period only if it is instituted early. There was a trend for the hyperbaric oxygen–ischemia plus reperfusion group to have the smallest infarcts; however, the Tukey’s test did not detect any significant differences among the infarct sizes in the three treated groups that showed protection.

Histopathological Evaluation

There was no difference in the risk volume between the two groups used for pathological evaluation. Histologically necrotic cells scattered among normal-appearing cells could be found in both groups, and these were scattered randomly from epicardium to endocardium throughout the risk region. In many instances, only a single or a small group of myocytes were necrotic. In other cases, a focus of necrotic cells formed a solid cluster. Such clusters could contain only a few cells or they could encompass as much as two thirds of the ventricular wall thickness. Necrotic cells had a distinct color difference in the Gomori aldehyde fuchsin trichrome stain and with hematoxylin and eosin were deeply eosinophilic with karyolysis. There was a distinct preponderance of single necrotic cells surrounded by normal myocardium in the hyperbaric oxygen group compared with the control group, in which the solid foci pattern of necrosis appeared to predominate. Quantitative evaluation of histological sections of two myocardial slices from each animal revealed that a reduced percentage of the risk region had become necrotic in the three hyperbaric oxygen hearts (7.7%, 10.3%, and 6.1%; mean, 8%) compared with that seen in the three control hearts (65.7%, 19.7%, and 34.5%; mean, 40%). These histological data agree closely with the infarct sizes revealed by tetrazolium in group 4, the hyperbaric oxygen–ischemia plus reperfusion group (9.8% necrosis), and in group 1, the ambient pressure control group (41.5% necrosis).

Discussion

The present data indicate that increased oxygen pressures administered during either the period of ischemia or reperfusion after acute ischemia decreases the extent of infarction in the rabbit heart compared with control animals breathing normobaric oxygen. That exposure to hyperbaric oxygen could protect the heart against infarction whether it occurred during the ischemic or early reperfusion period was an unanticipated finding.

Our data are congruent with those obtained by other investigators who found a protective effect of hyperbaric oxygen for varying durations up to 4 hours in canine hearts undergoing permanent occlusion.45 Dogs, unlike rabbits, are known to have an extensive collateral circulation, and it is likely that at least some protection derives from augmented blood oxygen content that would result in increased oxygen delivery to the ischemic zone by the collateral flow. Because long-term survival of ischemic myocardium depends on continuos nutrition, it is unlikely that a transient exposure to hyperbaric oxygen would have offered sustained benefit in a permanent occlusion model. The heart would be

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**TABLE 1. Hemodynamic Data for Preischemia, Ischemia, and Reperfusion in Experimental Animals**

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment Name</th>
<th>Preischemia</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR BP</td>
<td>HR BP</td>
<td>HR BP</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>209±11 69±07</td>
<td>193±17 60±06</td>
<td>192±15 59±05</td>
</tr>
<tr>
<td>2</td>
<td>Ischemia</td>
<td>174±05 58±03</td>
<td>177±06 48±13</td>
<td>176±06 56±03</td>
</tr>
<tr>
<td>3</td>
<td>Reperfusion</td>
<td>214±16 61±07</td>
<td>181±17 64±06</td>
<td>191±08 64±07</td>
</tr>
<tr>
<td>4</td>
<td>Ischemia plus reperfusion</td>
<td>187±08 63±05</td>
<td>176±07 65±04</td>
<td>164±04 54±04</td>
</tr>
<tr>
<td>5</td>
<td>Late reperfusion</td>
<td>182±22 66±07</td>
<td>159±12 52±03</td>
<td>159±08 70±23</td>
</tr>
<tr>
<td>6</td>
<td>Pressure control</td>
<td>189±10 60±03</td>
<td>191±10 60±04</td>
<td>175±09 51±03</td>
</tr>
</tbody>
</table>

HR indicates heart rate in beats per minute; BP, mean arterial blood pressure (mm Hg).

**TABLE 2. Group Characteristics and Infarct Data for Experimental Animals**

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental Conditions</th>
<th>No. of Animals</th>
<th>Heart Wt, g</th>
<th>Infarct Volume, cm³</th>
<th>Risk Volume, cm³</th>
<th>Infarct Size, % Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>7</td>
<td>6.4±0.36</td>
<td>0.17±0.05</td>
<td>0.54±0.11</td>
<td>41.5±4.59</td>
</tr>
<tr>
<td>2</td>
<td>Ischemia</td>
<td>15</td>
<td>7.1±0.30</td>
<td>0.13±0.03</td>
<td>0.76±0.06</td>
<td>16.2±2.68</td>
</tr>
<tr>
<td>3</td>
<td>Reperfusion</td>
<td>10</td>
<td>6.8±0.27</td>
<td>0.09±0.03</td>
<td>0.61±0.08</td>
<td>14.5±3.74</td>
</tr>
<tr>
<td>4</td>
<td>Ischemia plus reperfusion</td>
<td>9</td>
<td>7.5±0.49</td>
<td>0.07±0.03</td>
<td>0.70±0.13</td>
<td>9.8±2.68</td>
</tr>
<tr>
<td>5</td>
<td>Late reperfusion</td>
<td>8</td>
<td>7.6±0.35</td>
<td>0.40±0.08</td>
<td>1.07±0.11</td>
<td>35.8±3.71</td>
</tr>
<tr>
<td>6</td>
<td>Pressure control</td>
<td>6</td>
<td>7.6±0.69</td>
<td>0.45±0.03</td>
<td>1.00±0.06</td>
<td>44.9±2.52</td>
</tr>
</tbody>
</table>
expected to revert to the same ischemic state soon after the hyperbaric oxygen was withdrawn. In a reperfusion model, however, any improved oxygenation during the critical peri-ischemic period would delay the onset of cell death and the protection would be sustained with reperfusion. To that end, Thomas et al. \(^{15}\) examined the effect of hyperbaric oxygen on infarct size in a dog heart ischemia/reperfusion model. Although that study can be criticized for their failure to include collateral flow in their infarct size analysis, their results also indicate a strong oxygen-induced protective effect. Our study confirms their observation and extends it by examining the timing requirements for hyperbaric oxygen treatment.

In the present study, we used 2.5 atm absolute oxygen as our hyperbaric oxygen regimen because that pressure is commonly used in medical hyperbaric protocols. The control group was breathing oxygen at 1.0 atm absolute (ambient pressure), so there was only 1.5-atm difference between the groups. The effect of ventilating with room air versus 100% oxygen was recently examined in this model by Shiner et al. \(^{16}\) They found no difference in infarct size between the two groups. The pressure of oxygen used in the present study appears to be critical to the ischemic heart. Since only one level of hyperbaric oxygen was used, we do not know what minimum pressure of oxygen is required to produce protective effects.

Our data indicate that there is a critical time period in which hyperbaric oxygen must be administered to be protective and that hyperbaric oxygen was protective when started just before reperfusion. How hyperbaric oxygen at reperfusion was protective is not yet understood. During reperfusion there is a strong reactive hyperemia, during which time Po, in the tissue approaches that of the arterial blood, in this case 1900 mm Hg. We originally thought that hyperbaric oxygen at reperfusion would increase infarct size since oxygen-derived free radical–producing reactions would be accelerated by mass action. Paradoxically, however, we saw a decrease rather than an increase in infarction in these groups. We must conclude that the detrimental effects, if any, of increased production of free radicals at reperfusion are far outweighed by the beneficial effects of hyperbaric oxygen. Oxygen deprivation is known to damage mitochondrial structural integrity, and perhaps exposure to high oxygen pressures restores mitochondrial integrity, stimulates membrane function, or otherwise facilitates electron transport. \(^{17}\) Alternatively, or in addition, one may postulate that the increased oxygen might even have acted as a free radical quenching agent. \(^{18}\) Regardless of the mechanism, this finding could have clinical importance in that acute myocardial infarction patients could undergo hyperbaric oxygen as an adjunct to thrombolysis and thus be reperfused under hyperbaric oxygen conditions.

We found that hyperbaric oxygen was also protective when it was confined only to the ischemic period. By Henry’s law, we would expect the increased pressure to yield an additional 3.5 vol% of oxygen to be dissolved in the arterial blood, or about a 20% increase in arterial oxygen content. In species like the dog, enough residual flow is achieved to the ischemic zone via collateral vessels to delay cell death. \(^{1}\) Augmenting the oxygen content of the arterial blood by increasing the dissolved component with hyperbaric oxygen would indeed be expected to increase the effectiveness of the collateral flow. In a species like the rabbit, which lacks preformed collateral vessels, the collateral flow is insufficient to affect survival in the ischemic zone; thus, augmented oxygen delivery via collateral flow seems an unlikely explanation. Because the chest was opened, it might be argued that appreciable oxygen was diffusing directly into the ischemic zone from the epicardial surface. That is also unlikely because the chamber was pressurized with room air and oxygen was confined only to the inhaled gas. Some oxygen could have reached some of the ischemic myocardial tissue via diffusion across the endocardial surface, which is in contact with the arterialized blood in the ventricle, and from interfaces with normally perfused tissue. Only a few hundred micrometers of tissue could have been supplied by direct diffusion through the endocardium, however, and the rabbit’s ventricular wall is 3 to 4 mm thick.

One possible mechanism for delivering oxygen to the ischemic tissue is via the coronary venous level collateral circulation. \(^{19,20}\) In a previous study we showed that with occlusion of a coronary branch, a favorable pressure gradient is formed for venous blood from the normal zone to flow through anastomotic channels into the veins of the ischemic zone. Furthermore, because this occurs in small vessels and because the action of the heartbeat causes venous blood to ebb and flow retrogradely toward the capillary bed, some of this blood reaches exchange vessels in the ischemic zone. In the normal heart, venous level collateral flow is not nutritional because it has a low oxygen content. If the oxygen content of coronary sinus blood were augmented with hyperbaric oxygen, however, that blood flowing through venous level collaterals could be very nutritional.

Although it is currently not possible for us to sample coronary venous blood from the in situ rabbit heart, we did notice that during the periods of hyperbaric oxygen,
the epicardial cardiac veins appeared quite red, indicating high oxygenation. Whalen and Saltzman found that the effluent venous fluid from a working heart at 37°C and hyperbaric oxygen had a Po2 of well above 1300 mm Hg when the inflow had a Po2 of about 1400 mm Hg. Furthermore, the venous hemoglobin remained fully saturated in the hearts that were perfused with whole or diluted blood. Above 1.8 atm absolute O2, the percentage of the blood’s oxygen extracted by tissue is known to diminish in relation to the oxygen-carrying capacity, which causes a progressive decrease in the A-V difference.21

Dehydrogenase-catalyzed reduction of tetrazolium was used to visualize the infarcts in this study. Tetrazolium is reduced by NADH in the tissue to form a deep red formazan pigment, and the reaction is catalyzed by dehydrogenase enzymes, which are also in the tissue.22 If tissue has lost either enzyme or NADH, there will be no tetrazolium reduction and no staining of the tissue; therefore, the tissue can be considered unambiguously as dead. It has been shown that 2 to 3 hours of reperfusion in a nontreated animal will cause dehydrogenase and/or cofactor to wash out of nonviable cells so that the ultimate infarct size is revealed by tetrazolium at that time.23-25 The converse is not true in that tissue still retaining enzyme and cofactor is not necessarily alive. Some drugs may delay the washout of enzyme or cofactor such that nonviable tissue still stains as living even after 3 hours of reperfusion.26 Therefore, we must consider the possibility that hyperbaric oxygen might only be interfering with the ability of tetrazolium to differentiate between living and dead cells and that no actual limitation of infarct size had occurred. However, since free radical scavengers are normally associated with this artifact, it seems unlikely that a free radical promoter as was used here would induce the artifact as well. Another strong argument against hyperbaric oxygen treatment causing an artifact with the tetrazolium staining is the excellent agreement that we saw between the hearts evaluated with tetrazolium and those evaluated with histology. We agree that with only 3 hours of reperfusion, some tissue destined to infarct may not have been recognized as necrotic by either method; however, the agreement between the two methods was remarkable. The only way to exclude this possible artifact is to repeat the study using a much more complex recovery protocol that would allow the heart to be reperfused for several days. The extended reperfusion causes the histological pattern of infarction to become unambiguous. We are currently preparing to conduct such studies.

We conclude that hyperbaric oxygen therapy is capable of limiting infarction in the rabbit heart. Furthermore, the data indicate that application of hyperbaric oxygen at reperfusion can promote that salvage, suggesting that hyperbaric oxygen might be a useful adjunct to thrombolysis. The mechanism for this protection is currently a matter of speculation.

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