Studies of Amiodarone During Experimental Myocardial Infarction: Beneficial Effects on Hemodynamics and Infarct Size

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SUMMARY  The effect of amiodarone was investigated in a canine model of myocardial infarction. The left anterior descending coronary artery was occluded in 24 anesthetized dogs. After 15 minutes of coronary artery occlusion, the ischemic myocardium at risk of necrosis was determined by labeling the heart with technetium-99m-labeled human albumin microspheres injected into the systemic circulation through the left atrium, and the dogs were then randomized to either a saline-treated control group (n = 13) or an amiodarone-treated group (n = 11) that received i.v. amiodarone, 10 mg/kg, administered in a single bolus 30 minutes after coronary artery occlusion. Myocardial infarct size was determined directly after 6 hours of coronary occlusion by incubation of sections of myocardium in triphenyltetrazolium chloride, a dehydrogenase stain, and expressed as a percentage of left ventricle below occlusion. Autoradiography of the stained myocardial sections was performed to determine the ischemic myocardium at risk of necrosis, which was similar in the control and amiodarone-treated groups (31.8 ± 2.8% vs 32.5 ± 3.3% of the left ventricle, respectively). In the amiodarone-treated group, only 67.1 ± 8.4% of the myocardium at risk became necrotic; in the control group, 97.5 ± 7.7% of the myocardium at risk became necrotic (p < 0.01), representing 21.8 ± 3.5% vs 31.1 ± 2.8% of the left ventricle below occlusion, respectively (p < 0.025). Amiodarone decreased heart rate, contractility and afterload. Its beneficial action on infarct size is related presumably to reduced myocardial oxygen demand.

AMIODARONE, a benzofuran derivative, is useful in the treatment of chronic angina pectoris.1-3 Amiodarone is also an effective antiarrhythmic agent, especially for supraventricular arrhythmias in the presence of Wolff-Parkinson-White syndrome and for ventricular arrhythmias.4-10 Amiodarone also decreases coronary and systemic vascular resistance without reducing cardiac output or augmenting myocardial oxygen consumption.11-13 We investigated the effects of amiodarone on infarct size after experimental coronary artery occlusion. An autoradiographic method was used to determine the myocardium at risk of necrosis, using technetium-99m-labeled human albumin microspheres to identify ischemic myocardium before an intervention.14 Another determination of ischemic myocardium at risk of necrosis was made after treatment.15 Myocardial infarct size was determined directly using triphenyltetrazolium chloride, a marker for dehydrogenase enzymes.

Materials and Methods

Thirty-one adult mongrel dogs, mean body weight 20 ± 2 kg, were anesthetized with i.v. sodium thiopental, 20 mg/kg, intubated, and ventilated with room air using a Harvard respirator. The left jugular vein and the left common carotid artery were cannulated. Arterial pressure was monitored with a Statham P23Db pressure transducer, and recorded with lead aVf of the ECG on a Brush multichannel recorder.

A left thoracotomy was performed and the heart was suspended in a pericardial cradle. The left atrial appendage was cannulated. The left anterior descending coronary artery was dissected free of adventitia and occluded at a standardized location proximal to the first major apical diagonal branch, using a Schwartz arterial clamp. Hearts that failed to develop apical cyanosis were not used. Lidocaine hydrochloride, 2 mg/kg i.v., was administered prophylactically to all dogs immediately before coronary occlusion. Ischemic myocardium at risk of necrosis was identified using an autoradiographic method.14 Fifteen minutes after coronary artery occlusion, 600,000 human albumin microspheres (3M Inc.) were injected into the left atrial cannula. These microspheres had been labeled with 0.5 mCi/kg of technetium-99m and had been vigorously agitated and ultrasonicated for 10 minutes. Labeling efficiency was determined by micropore filtration and was in excess of 99%.

The dogs were randomized into control (n = 13) and treated (n = 11) groups. The control group received 1 liter of normal saline intravenously during the experimental period (6 hours). The treated group received, in addition to 1 liter of normal saline, 10 mg/kg of amiodarone, administered 30 minutes after coronary occlusion. The amiodarone powder (LaBaz, Inc.) had been dissolved in a small volume of heated, distilled water and was administered over 3 minutes by i.v. infusion.

Six hours after coronary artery occlusion, a second determination of ischemic myocardium at risk of necrosis was performed with a left atrial injection of a fluorescent dye, thioflavin-S. This dye stains endothelial cells in well-perfused myocardium, but does not

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stain the endothelium of poorly perfused myocardium. The dogs were then sacrificed with an overdose of thiaymal and the hearts removed. The left ventricle was dissected free of the great vessels, atria and right ventricle. To stiffen the ventricle for easier sectioning, the left ventricular surface and cavity were lightly sprayed with Freon 12. This procedure freezes only the surface of the ventricle. Transverse left ventricular sections 3 mm thick were made parallel to the atrioventricular groove using an electric rotary blade. Each section was weighed and, using magnification, traced under ultraviolet light. Myocardium that received low flow (at risk of necrosis) after treatment was nonfluorescent; myocardium that was normally perfused fluoresced yellow green. The sections were then incubated in a phosphate-buffered 1% solution of triphenyltetrazolium chloride (TTC) at 37°C for 15 minutes. TTC stains viable, dehydrogenase-enzyme-containing myocardium brick red, while necrotic myocardium, which is depleted of dehydrogenases, is unstained and appears pale gray. This stain is a reliable indicator of myocardial necrosis. Incubation in the stain did not significantly alter left ventricular weight. Normal and infarcted areas of myocardium were traced on plastic sheets, again using magnification.

Autoradiography was performed by arranging the left ventricular sections on high-speed x-ray film in an image-amplifying cassette and exposing the film for 18 hours at 2°C. The film was developed in an X-Omat processor (Kodak Medical Products). Using this technique, areas of normal perfusion appear as areas of increased radiographic density, while ischemic areas appear as clearly demarcated areas of diminished density. Tracings of the ischemic and normal myocardium were then made under magnification. Planimetry was performed, and the volume of ischemic, infarcted and normal myocardium determined using myocardium at risk before and after treatment and event myocardial necrosis. Extent of myocardial necrosis and ischemic myocardium at risk were expressed as a percentage of the total left ventricular volume distal to the occlusion and as a percentage of the myocardium at risk of necrosis as determined by the autoradiographic technique.

In seven other dogs, the acute hemodynamic effects of amiodarone were studied. These dogs were prepared in a similar fashion, but with a Millar micromanometer-tip catheter inserted into the apex of the left ventricle to record left ventricular systolic and end-diastolic pressures. Left ventricular end-diastolic pressure was determined at the end of the a wave, dP/dt was determined at a developed pressure of 40 mm Hg and maximum negative dP/dt was also determined. Amiodarone, 10 mg/kg, was infused 15 minutes after coronary occlusion. Hemodynamics were recorded 5 minutes before coronary occlusion, immediately before amiodarone and 2 minutes after amiodarone.

The results were analyzed by paired and unpaired t test as appropriate. All results are expressed as mean ± SEM.

**Results**

Control and treated dogs did not differ significantly in regard to body weight, preocclusion heart rate or mean arterial pressure. The distance from the coronary ostium to the site of occlusion ranged from 2.5–3.0 cm in both groups. There were no significant hemodynamic changes after infusion of labeled human albumin microspheres in either group. Coronary artery occlusion resulted in significant reductions in left ventricular systolic and mean aortic pressures, as well as in dP/dt at 40 mm Hg and in peak negative dP/dt, while left ventricular end-diastolic pressure rose (table 1). In the saline-treated group, there were no significant hemodynamic changes from 15 minutes to 6 hours after coronary artery occlusion.

The quantity of myocardium at risk of infarction was similar in the control and amiodarone-treated groups (31.8 ± 2.8% vs 32.5 ± 3.3% of the left ventricle [NS]) (figs. 1–3). The second measurement of the quantity of ischemic myocardium at risk was made at the end of the treatment period using thioflavin-S. In

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**TABLE 1. Mean Hemodynamic Values in the Treated Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion</th>
<th>Before treatment (15 min after occlusion)</th>
<th>After treatment (17 min after occlusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>154 ± 4</td>
<td>154 ± 6</td>
<td>119 ± 6*</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>126 ± 7</td>
<td>112 ± 7</td>
<td>96 ± 7*</td>
</tr>
<tr>
<td>LV peak pressure (mm Hg)</td>
<td>143 ± 7</td>
<td>129 ± 8</td>
<td>113 ± 7*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>6.7 ± 1.7</td>
<td>11.3 ± 1.5</td>
<td>11.3 ± 1.7</td>
</tr>
<tr>
<td>LV dP/dt at 40 mm Hg (mm Hg)</td>
<td>2510 ± 300</td>
<td>2340 ± 300</td>
<td>1320 ± 200*</td>
</tr>
<tr>
<td>Max negative dP/dt (mm Hg/sec)</td>
<td>3440 ± 500</td>
<td>3000 ± 500</td>
<td>1860 ± 400*</td>
</tr>
</tbody>
</table>

*P < 0.01 vs pretreatment.

Abbreviation: LV = left ventricular.
FIGURE 1. (A) Autoradiogram of a midventricular section from an amiodarone-treated dog. Perfused tissue appears white; ischemic tissue appears as a dark region of reduced radioactivity. (B) The same section of myocardium photographed under ultraviolet light. Tissue perfused with thioflavin-S is fluorescent; ischemic tissue is nonfluorescent. (C) The same section after triphenyltetrazolium chloride staining. Infarcted tissue is unstained (light gray); noninfarcted myocardium stains darkly. (D) Diagrammatic representation of the same section.

In the control group, the quantity of myocardium at risk at this time did not differ from that determined early in the course of myocardial ischemia by autoradiography (31.8 ± 2.8% [early] vs 31.9 ± 2.8% [late] of the left ventricle, respectively). In contrast, amiodarone reduced the quantity of myocardium at risk (32.5 ± 3.3% [early] vs 28.7 ± 3.5% [late]) (p < 0.05).

Infarct size, determined after 6 hours of occlusion and expressed as a percentage of the left ventricle below occlusion, was significantly smaller in the amiodarone-treated group than in the control group (21.8 ± 3.5% vs 31.1 ± 2.8% of the left ventricle, respectively, p < 0.025) (fig. 3). Infarct size was also calculated as the ratio of myocardial necrosis to myocardium initially at risk of necrosis, i.e., as determined by autoradiography. In the control group, 97.8 ± 5.7% and in the treated group only 67.1 ± 8.4% of the myocardium at risk became necrotic (p < 0.001).

In the seven dogs in which the hemodynamic effects of amiodarone after acute coronary occlusion were...
FIGURE 2. Mid-left ventricular sections from a control dog and from an amiodarone-treated dog. The myocardium at risk and the actual necrosis that has developed are similar in the saline-treated control dog. Although both hearts had similar quantities of myocardium at risk, there was less necrosis in the heart of the amiodarone-treated dog.

determined, heart rate, mean aortic pressure, peak left ventricular systolic pressure, dP/dt at 40 mm Hg and maximum negative dP/dt all decreased. Left ventricular end-diastolic pressure remained constant (table 1).

Discussion

This study demonstrates that amiodarone exerts a significant protective effect on ischemic myocardium, reducing myocardial infarct size substantially after coronary artery occlusion in the dog. This effect was demonstrable when infarct size was expressed as a fraction of the left ventricle, but was even more readily apparent when infarct size was expressed as a fraction of ischemic myocardium at risk of necrosis. Amiodarone is clinically effective in the treatment of angina pectoris, an effect which is thought to result from both a direct coronary vasodilating effect and from noncompetitive antiadrenergic blocking activity, and the reduced release of neurotransmitter from the presynaptic adrenergic neurons. Amiodarone exerts other actions that might be beneficial to ischemic myocardium because, like its antiadrenergic actions mentioned above, they tend to reduce myocardial oxygen consumption. These actions include reducing afterload and heart rate and decreasing contractility (table 1). Although the decrease in contractility could be a disadvantage in the setting of ischemic heart disease, it is balanced by afterload reduction and does not alter the left ventricular end-diastolic pressure (table 1).

We used an autoradiographic method of determining the myocardium at risk of necrosis. In the canine model of myocardial infarction, differences in distribution of coronary vessels and in collaterals result in a variation in the volume of myocardium at risk of necrosis after occlusion of the coronary artery at a standardized location. Several methods have been introduced to correct for this variation. Among these is the postmortem infusion of dyes or of barium gels into the occluded and nonoccluded coronary arteries with a balanced perfusion pressure. These methods outline the anatomic borders of the perfusion bed of the occluded coronary artery. The in vitro Monastral blue technique perfused myocardium at risk below the site of occlusion at aortic pressure. Since the in vivo perfusion pressure in the occluded coronary artery is lower than that in the nonoccluded coronary system, the in vitro dye techniques include myocardium in the region that is protected from necrosis by collateral flow. Similarly, when barium gel was injected proximal to the site of circumflex coronary artery occlusions, the region at risk also exceeded the region of necrosis and included a larger border zone of myocardium within which ischemia was insufficient to result in cell death. The in vivo methods used in the present study delineate a zone of myocardium smaller than the anatomic coronary bed size in which ischemia is more severe and hence, a larger percentage becomes necrotic.

The autoradiographic method has the advantage of delineating ischemic myocardium before an intervention. Careful control of the amount of label and the duration of autoradiographic exposure as well as film

FIGURE 3. The effects of amiodarone on the extent of myocardial necrosis. Control and amiodarone-treated dogs had similar quantities of myocardium at risk of necrosis as determined by autoradiography and expressed as percentage of left ventricle below the site of occlusion. Infarct size, again expressed as a percentage of the left ventricle below occlusion and determined by triphenyltetrazolium staining, was smaller in the amiodarone-treated group. Similarly, amiodarone reduced infarct size, expressed as the ratio of necrosis to the myocardium at risk as determined by autoradiography.
development and the uniformity of section thickness are all required to obtain autoradiograms of adequate density and resolution. Thus, the myocardium determined to be at risk by this method when the radioactive microspheres are injected 15 minutes after coronary occlusion before the intervention does not reflect any changes in collateral blood flow that might be induced by the intervention. Moreover, although collateral flow can change markedly during the first minutes after a coronary occlusion in untreated animals, after 15 minutes of occlusion, the pattern of collateral flow has stabilized. The administration of thioflavin S into the left atrium after coronary occlusion is also useful for identifying ischemic myocardium. The nonfluorescent myocardium is severely ischemic, with flows less than 0.4 ml/g/min. The quantity of myocardium at risk of necrosis when this method was applied 6 hours after coronary artery occlusion was lower than that found to be ischemic before treatment with amiodarone, indicating that this drug decreases the volume of myocardium at risk of necrosis, presumably by increasing the collateral blood flow to the ischemic zone.

This study demonstrates that amiodarone can provide significant protection to ischemic myocardium. Although this drug has a very high LD50 for rats when given orally (5 g/kg), and there is little evidence that amiodarone has caused death in humans in the face of extensive clinical use outside of the United States, this drug causes significant side effects. These include the development of asymptomatic corneal microdeposits, which are reversible after the drug is discontinued. Photosensitivity reactions and gastrointestinal symptoms are common. In addition, hypo- and hyperthyroidism occasionally develop. Despite these side effects, the combination of potent antiarrhythmic and antianginal actions, combined with its ability, as demonstrated in this study, of limiting infarct size after experimental coronary occlusion, suggest that amiodarone can be of considerable clinical value in the treatment of patients with ischemic heart disease.

Acknowledgment

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References

22. Reimer KA, Jennings RB: The waveform phenomena of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 40: 633, 1979
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