EXPERIMENTAL STUDIES

Effect of Verapamil on pH of Ischemic Canine Myocardium

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Verapamil has been shown to depress the contractility of ischemic myocardium. The present study was designed to determine whether that effect is due to an increase in ischemic injury caused by the drug or whether it might reflect a protective effect. A critical partial occlusion was effected on the left anterior descending coronary artery of 16 open chest foxhounds. A fiberoptic pH probe was implanted in the subendocardium of the ischemic zone, and coronary blood flow was reduced by 79% from a control value of 38 ± 4 ml/min and held constant. Mean coronary perfusion pressure was decreased 48% from its control value of 90 ± 10 mm Hg and remained constant.

Eight animals were treated with intravenous verapamil, beginning 20 to 30 minutes after the onset of ischemia, in incremental doses (5, 10 and 20 µg/kg per min) and eight were treated with placebo. The pH of the ischemic zone increased after institution of treatment in the verapamil group (+ 0.04 ± 0.05 pH unit) whereas it decreased in the placebo group (− 0.06 ± 0.4 pH unit) during the first dose (p < 0.05). Although the difference in pH between the two groups was marked at all doses (p < 0.03) compared with control partial occlusion, verapamil caused no significant change in heart rate (+ 0.1 ± 1 beat/min in the verapamil group versus + 0.6 ± 4.5 beats/min in the placebo group), mean arterial pressure (− 7.5 ± 4 versus − 4.3 ± 3 mm Hg, respectively) or cardiac output (− 0.2 ± 0.07 versus − 0.02 ± 0.04 liters/min, respectively) comparing control with the first or the second dose of verapamil. The third dose of verapamil caused large decreases in heart rate (29%) and mean arterial pressure (23%) and caused conduction block in most animals. Improvement of myocardial acidosis after 20 to 30 minutes of ischemia as a result of verapamil infusion confirms that verapamil exerted a beneficial effect on ischemic myocardium in this experimental model.

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flow (peak ejection rate) as an index of left ventricular function (5). A catheter was placed in the right femoral artery for measurement of phasic and mean aortic blood pressures. Pressures were monitored by Statham P23Db pressure transducers. A side hole catheter was inserted into the left atrium and secured with a pursestring suture for measurement of left atrial pressures.

The left anterior descending coronary artery was dissected proximally. Heparin (10,000 U) was injected intravenously and the left anterior descending coronary artery was cannulated with a perfusion system connecting the left carotid artery to the left anterior descending coronary artery proximal to the first diagonal branch. The perfusion system consisted of Tygon tubing (6 mm internal diameter) equipped with the following: a screw clamp for subsequent partial coronary occlusion, a port between the constrictor and the cannulating site for coronary perfusion pressure measurements and a Y connection. The latter incorporated a blood-calibrated electromagnetic flow probe (Carolina Medical Electronics) to permit uninterrupted perfusion of the cannula-dependent coronary artery bed during balancing the flowmeter at zero mechanical flow (Fig. 1). The left anterior descending coronary artery was ligated proximally and its distal portion was quickly cannulated with a 12 gauge blunt needle and perfused with arterial blood through the perfusion circuit. Heparin (6,000 U) was given intravenously at 30 minute intervals to insure cannula patency.

**Determination of pH.** Intramyocardial pH was measured by a miniature fiberoptic probe system which has been previously described (2–4). In vivo validation was performed in separate experiments (6). Briefly, the probe consists of two optical fibers (0.075 mm in diameter) and a 0.12 mm diameter semipermeable membrane containing a pH-sensitive dye housed in a modified 25 gauge (0.5 mm outer diameter) stainless steel needle to facilitate implantation. The two internally reflecting optical fibers are of sufficient length that the illumination and photodetection support module and computer system can be offset from the experimental field.

The pH measurement system was calibrated at 37°C with buffers of pH 6.840 and pH 7.384. In 13 animals, a single fiberoptic probe was implanted in the subendocardium (depth 5 to 8 mm) of the potentially ischemic zone and secured by sutures. In the last three experiments, multiple fiberoptic probes were available for implantation. Four miniature fiberoptic pH probes were implanted and secured in both the normal zone and potentially ischemic zone at depths below the epicardial surface of 3 to 4 mm (subepicardium) and 5.5 to 8 mm (subendocardium). The two probes in each zone were implanted within 5 to 10 mm of each other and position was confirmed postmortem. A fifth fiberoptic probe was implanted within the left ventricular cavity for continuous on-line determination of arterial blood pH (Fig. 1). The readings of arterial blood pH measurements obtained by the fiberoptic probe were confirmed by arterial blood gas determinations using a glass electrode (Instrumentation Laboratories). The temperature of the pericardial fluid was maintained in the physiologic range and monitored by means of two temperature probes placed in the rectum and pericardial sac.

**Experimental protocol.** After control measurements, a critical coronary artery stenosis was effected by means of an adjustable screw clamp on the carotid-left anterior descending perfusion system. This was done by holding the mean coronary perfusion pressure at 50 mm Hg and the coronary blood flow at approximately 20% of control, such that the reactive hyperemic response to temporary occlusion was abolished (7). The temporary total occlusion of 15 seconds' duration was effected by means of a surgical clamp on the perfusion tubing. With total occlusion, coronary blood flow and coronary perfusion pressure both decreased to zero. With release of the total occlusion, the coronary blood flow returned to its control partial occlusion level. Similarly, the mean diastolic perfusion pressure returned to its control level (31 ± 13 mm Hg). Data obtained during partial occlusion included measurements of pressures and blood flows and continuous values of pH.
Verapamil (n = 8) or normal saline placebo (n = 8) was administered in a randomized fashion to different dogs as an intravenous infusion in three incremental doses (5, 10 and 20 μg/kg per min) using a Harvard infusion pump after an initial bolus of 30 μg/kg administered over 2 minutes. Similar intravenous infusion rates have been employed clinically in our institution in catheterization laboratory studies designed to demonstrate a hemodynamic effect of the drug (8). Infusion was begun only after the lower pH was stable for at least 3 to 5 minutes. Hemodynamic measurements were taken at 5 minute intervals. Each dose of drug or placebo was administered during a 20 to 30 minute period, and the infusion was continued until the next dose was initiated. Although the length of the experiment was mildly variable depending on the total time of data acquisition, the comparison of the placebo group followed up for similar lengths of time provides a control for any changes of the experimental preparation over time. Serum verapamil levels were determined by the method of JAONI et al. (9).

After completion of data acquisition on the third dose of verapamil or placebo, the animals were killed by injection of an overdose of pentobarbital. After each experiment, reliability of fiberoptic pH measurement was confirmed by accurate reading of the standardization buffers.

To confirm the location of the pH probes in the ischemic zone supplied by the left anterior descending coronary artery, we identified ischemic myocardium by injection of Evans blue dye into the perfusion cannula. We confirmed that the probe or probes were located at least 15 mm inside unstained tissue (II). This procedure minimizes contamination of ischemic myocardium by overlapping normal zone tissue (10,11) because this overlap occurs almost exclusively within the first 5 mm inside the border between blue and unstained tissue (11).

Statistics. We calculated mean ± standard error for group data and tested the significance of differences by the Student’s t test for paired data or for independent samples and analysis of variance and covariance as appropriate (12). Differences were considered significant when p < 0.05.

Results

Baseline pH and hemodynamic data for both treatment and control groups show no significant differences between those animals given verapamil and those given placebo either in the preocclusion state or after partial occlusion (Table 1). Comparison of the differences between the preocclusion versus partial occlusion within the same group is shown by the p value.

Myocardial pH. The pH data from a representative experiment are shown in Figure 2. The initial small decrease in pH recorded over the first 30 minutes from pH 7.50 to 7.44 is due to respirator adjustments to achieve a normal arterial blood pH. The pH of the ischemic zone decreased from 7.43 to 7.24 by 10 minutes after partial coronary artery occlusion, whereas the pH of arterial blood and of the normally perfused myocardium were unaffected. After 50 minutes of partial coronary artery occlusion, the pH in the ischemic zone was 6.90. The pH was stable in the range of 6.92 to 6.90 for 5 minutes before the initiation of verapamil infusion. After continued infusion of verapamil in incremental doses, pH increased toward the level of the normal zone pH. The pH of arterial blood and of the normal zone did not change under the influence of verapamil. With ventricular fibrillation, all probes recorded precipitous decreases in pH, confirming the continued ability of the probes to respond to changing hydrogen ion concentrations.

Ischemic zone pH increased in the verapamil-treated dogs compared with the control group (Fig. 3). The effect was

### Table 1. Summary of Results*

<table>
<thead>
<tr>
<th>Group</th>
<th>pH</th>
<th>HR</th>
<th>MAP</th>
<th>CBF</th>
<th>CPP</th>
<th>CPP_d</th>
<th>PER</th>
<th>LAP</th>
<th>CO</th>
<th>PVR</th>
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<tr>
<td><strong>A. Treatment (VRP)</strong></td>
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<td></td>
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<tr>
<td>Preocclusion</td>
<td>7.46 ± 0.02</td>
<td>135 ± 7</td>
<td>86 ± 5</td>
<td>42 ± 6</td>
<td>85 ± 6</td>
<td>71 ± 4</td>
<td>4.8 ± 0.4</td>
<td>5 ± 1</td>
<td>1.2 ± 0.1</td>
<td>75 ± 12</td>
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<tr>
<td>(n)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
<td>(4)</td>
<td>(8)</td>
<td>(6)</td>
<td>(7)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Partial occlusion</td>
<td>7.11 ± 0.05</td>
<td>127 ± 9</td>
<td>77 ± 5</td>
<td>9 ± 1</td>
<td>46 ± 3</td>
<td>31 ± 3</td>
<td>4.4 ± 0.08</td>
<td>6 ± 1</td>
<td>1.2 ± 0.1</td>
<td>72 ± 9</td>
</tr>
<tr>
<td>(n)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
<td>(7)</td>
<td>(7)</td>
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<td>(4)</td>
<td>(7)</td>
<td>(5)</td>
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<tr>
<td>p Value</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.035</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td><strong>B. Control (placebo)</strong></td>
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<tr>
<td>Preocclusion</td>
<td>7.42 ± 0.02</td>
<td>150 ± 9</td>
<td>99 ± 6</td>
<td>34 ± 4</td>
<td>95 ± 8</td>
<td>78 ± 6</td>
<td>3.9 ± 0.3</td>
<td>4 ± 2</td>
<td>1.1 ± 0.1</td>
<td>94 ± 10</td>
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<tr>
<td>(n)</td>
<td>(8)</td>
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<td>(8)</td>
<td>(8)</td>
<td>(5)</td>
<td>(8)</td>
<td>(5)</td>
<td>(8)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Partial occlusion</td>
<td>7.18 ± 0.05</td>
<td>151 ± 8</td>
<td>92 ± 6</td>
<td>7 ± 1</td>
<td>38 ± 4</td>
<td>29 ± 5</td>
<td>3.5 ± 0.1</td>
<td>4 ± 2</td>
<td>0.9 ± 0.1</td>
<td>103 ± 7</td>
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<td>(5)</td>
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<td>(4)</td>
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<td></td>
</tr>
<tr>
<td>p Value</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
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<td>NS</td>
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*Values are expressed as mean ± SEM. †There are no statistically significant differences (by Student’s unpaired t test) between Groups A and B preocclusion or between Groups A and B after partial coronary artery occlusion. CBF = mean coronary blood flow (ml/min); CO = cardiac output (liters/min); CPP = mean coronary perfusion pressure (mm Hg); CPP_d = diastolic coronary perfusion pressure (mm Hg); HR = heart rate (beats/min); LAP = mean left atrial pressure (mm Hg); MAP = mean arterial pressure (mm Hg); PER = peak ejection rate (liters/min); PVR = calculated peripheral vascular resistance (mm Hg/liter per min); VRP = verapamil.
particularly apparent with the first (5 μg/kg per min) and the second (10 μg/kg per min) doses, although all three doses resulted in a higher pH compared with placebo-treated dogs (p < 0.03 by analysis of covariance). While the third dose of verapamil reduced aortic pressure, it actually caused a decrease in pH, but this decrease was not as great as the decrease in the placebo-treated group. Control values of intramyocardial pH before verapamil infusion were actually lower (Fig. 3A), suggesting more severe ischemia in the verapamil treatment group (pH 7.11 ± 0.05) compared with the control group (pH 7.18 ± 0.05), but the difference was not significant (Table 1). Individual data points are presented in Figure 4, which shows a group trend of improved pH (range 0.16 to 0.31 pH units, mean 0.22 ± 0.04) of ischemic myocardium during verapamil infusion.

**Determinants of myocardial oxygen supply and demand.** Coronary blood flow (Fig. 5A) was reduced to 21% of control (p < 0.01) by partial coronary occlusion in the treatment group and to 20% of control (p < 0.001) in the control group (Table 1). The difference between the two postpartial occlusion measurements is not significant. Coronary blood flow was held constant during three doses of verapamil and measured 6 ± 1, 9 ± 1 and 9 ± 1 ml/min with each dose of verapamil compared with 9 ± 2, 7 ± 2 and 8 ± 4 ml/min with each dose of placebo (Fig. 5A). Analysis of covariance confirmed no difference in coronary blood flow between the two groups. Coronary perfusion pressure distal to the partial occlusion (Fig. 5B) decreased with partial occlusion (90 ± 5 versus 43 ± 3 mm Hg; p < 0.03), but it did not decrease further during verapamil infusion.

Figure 6A demonstrates a marked dose-related decrease in heart rate in the verapamil-treated group. The third dose of verapamil (20 μg/kg per min) had profound negative chronotropic and dromotropic effects. Conduction disturbances occurred in all dogs in the verapamil group and
included sinus bradycardia, first and second degree atrioventricular (AV) block (Mobitz type I) and complete heart block in one dog. Only mild increases in the PR interval were seen with the first and second doses. Analysis of covariance confirmed no difference in heart rate between the verapamil-treated group and the placebo group for the first and second doses, although the decrease was significant for all three doses (p < 0.04). Verapamil dosage was linearly related to serum verapamil levels. The adequacy of the loading dose was apparent because serum levels after the loading dose approximated those achieved with the initial dose of verapamil. Although verapamil caused a significant reduction in mean arterial pressure, considering all three doses (p < 0.02), the changes were not significant with the first two doses of verapamil employed.

Several indexes of left ventricular function decreased progressively with increasing doses of verapamil. When assessed by one way analysis of variance, cardiac output (Fig. 7B) (p < 0.04) and peak ejection rate (Fig. 7C) (p < 0.04) decreased significantly. Analysis of variance showed no significant difference in left atrial pressure between the control and treatment groups (Fig. 7A), but the relation between left atrial pressure and cardiac output indicated diminished cardiac function with verapamil (Fig. 8). This representation of the Starling relation demonstrates that the ventricular function curve is depressed by verapamil.

**Discussion**

**Protective effect of verapamil in ischemia.** Verapamil is clinically effective in the treatment of ischemic heart disease (13–16). In theory, the drug may favorably affect both oxygen supply and demand. A previous study (1) showed that verapamil selectively depresses the contractile function of ischemic myocardium; that is, doses that exert no effect on contractile function of normal zone myocardium significantly diminish contractile function of ischemic tissue. A more recent study from our laboratory (17) demonstrated reduced global and regional function with verapamil treatment in awake animals with partial coronary occlusion. By
documenting that verapamil treatment improves pH of ischemic myocardium, our study suggests that the depression of cardiac function is a primary drug effect that results in amelioration of ischemia-induced acidosis, rather than a secondary drug effect resulting from a worsening of ischemic injury to the myocardium. This beneficial effect to depress cardiac function might be viewed as if the drug were "splinting" the ischemic myocardium, thereby reducing local myocardial oxygen demand and diminishing ischemia in the jeopardized region.

If intramyocardial pH is considered as an indicator of the balance between myocardial oxygen supply and demand in the presence of myocardial ischemia, then one can assess the mechanism by which verapamil might improve pH. Other experiments in this laboratory show a good correlation between intramyocardial pH and the reduction in coronary blood flow (r = 0.80), myocardial concentration of lactate (r = 0.88) and adenosine triphosphate (r = 0.78) (Ro and Patterson, unpublished observations). Oxygen delivery to the ischemic myocardium was held constant by design in these experiments to assess effects on oxygen demand and ischemic injury. We employed three doses of verapamil in an attempt to separate the multiple potential effects of this calcium channel blocking agent. Verapamil decreased heart
Figure 8. Effect of verapamil on left ventricular function (Starling curve). The percent change in cardiac output of verapamil-treated (solid triangles) and placebo-treated (solid circles) animals is shown graphed against the mean left atrial pressure (LAP). The data points are designated for each of the three incremental doses by an arabic numeral associated with the letter V (for verapamil-treated) or P (for placebo-treated). This representation of the Starling relation indicates a depression of cardiac output at the same or higher values of left atrial pressure with verapamil (p < 0.05 by analysis of variance).

rate, aortic pressure, cardiac output and peak ejection rate when all doses were considered. At the two lowest doses, however, verapamil improved the pH of the ischemic myocardium without significantly affecting the heart rate or mean aortic pressure (Fig. 6). An improvement in pH was apparent with the first dose of verapamil before any hemodynamic change, suggesting that the protective effect of verapamil is independent of the indexes of myocardial oxygen demand or supply measured in this study. It is quite possible that ischemic regional function had decreased because this result was found using similar doses in an earlier study from our laboratory (17). Another study from our laboratory (1) indicated that verapamil selectively depressed contractility of ischemic myocardium to diminish myocardial oxygen demand in the ischemic region without significant effect on global function.

Critique of methods. Our conclusion that verapamil improved pH of ischemic myocardium seems justifiable by the methods employed and results obtained. The selection of the open chest model was dictated by technical considerations relating to implantation of the flexible fiberoptic pH probe. We employed a model of critical partial coronary occlusion to stimulate angina pectoris resulting from moderate transient ischemia. Because the stenosis was effected to eliminate the reactive hyperemic response and reduce coronary blood flow, the ischemic bed in this model was presumably maximally dilated. The beneficial verapamil effect was not due to any coronary vasodilator effect because coronary blood flow was held constant by design (Fig. 5A). An acute partial coronary occlusion does not present much stimulus to collateral flow because the coronary pressure distal to the obstruction does not fall far enough below coronary pressure at the origin of the collateral vessels to provide a high drive pressure gradient for collateral myocardial blood flow (18). In support of this interpretation, a previous study from our laboratory (19) showed that verapamil had no effect on collateral blood flow. We did not measure an increase in peripheral coronary pressure, which should have increased if collateral vessels had delivered more flow (Fig. 5C) (18). In addition, by holding coronary flow constant and observing a constant coronary artery pressure and left atrial pressure, it is unlikely that changes in transmural distribution of blood flow were causing major changes in tissue blood flow because the transmural gradient of pressure between coronary artery and left ventricular filling pressure was constant (20).

Possible mechanisms. We were able to demonstrate a beneficial effect of verapamil treatment even though treatment began 20 to 30 minutes after critical partial coronary artery occlusion. This represents an important finding in that it indicates that verapamil can lessen ongoing ischemic myocardial injury. Sherman et al. (21) also demonstrated a beneficial effect of verapamil on ischemic canine myocardium independent of heart rate or myocardial blood flow. This protective effect on the ischemic zone was evidenced by preserved postextrasystolic potentiation, an index of the latent contractile response, in the verapamil-treated animals. Regional systolic shortening in the ischemic zone was abolished equally in control and verapamil-treated dogs, but postextrasystolic potentiation was preserved only in verapamil-treated dogs (21). The possibility that verapamil improved pH by direct blockade of calcium entry into ischemic
cells (22,23) cannot be confirmed or refuted by our experiment, but this possibility is not necessary to explain the results of our study.

Because these animal studies were performed in dogs having no evidence of heart failure, it is possible that verapamil-induced reduction of left ventricular function might not improve pH in the setting of left ventricular failure. Decreased left ventricular function might increase wall tension and oxygen demands in ischemic myocardium in the failing heart and lead to an opposite effect of verapamil on pH. Thus, it should not be assumed that verapamil improves myocardial pH in all settings of acute myocardial ischemia.

Conclusions. Our study demonstrates a beneficial effect of verapamil on myocardial ischemic injury in an experimental model designed to represent fixed atherosclerotic lesions where there would be no element of coronary spasm involved. This beneficial effect was observed even though treatment was not started until several minutes after the onset of ischemia. The favorable effects of verapamil on pH of ischemic myocardium were associated with no change in coronary hemodynamic measurements distal to the partial coronary occlusion, indicating that the favorable effects could not be attributed to improved tissue blood flow. This improvement in pH was evident during the lowest dose before systemic hemodynamic changes were apparent. These findings with verapamil in an animal model of myocardial ischemia offer objective evidence of improved metabolism of ischemic myocardium and may represent one of the mechanisms responsible for the antianginal effect of verapamil observed in patients.

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References