Comparative Effect of Verapamil and Nitroglycerin on Collateral Blood Flow

ROBERT FORMAN, M.D., CALVIN ENG, M.D., AND EDWARD S. KIRK, PH.D.

SUMMARY The effects of intracoronary verapamil and nitroglycerin on collateral blood flow were compared under conditions where coronary perfusion pressure was held constant with a servopump and the systemic effects of the drugs were minimal. Both drugs were infused into 12 anesthetized dogs after occlusion of the left anterior descending coronary artery (LAD) and regional myocardial blood flow (MBF) was measured using microspheres. Before the LAD occlusion, the myocardium not perfused by the LAD was labeled to permit calculation of the fraction of tissue normally perfused in the LAD samples and corrections for collateral flow. The central ischemic zone contained 2.5 ± 0.3% normally perfused myocardium and a 4-mm border zone contained 26.8 ± 4.3% normal myocardium. This border zone contained 10% of the total tissue supplied by the LAD. The MBF in the central ischemic zone increased from 0.101 ± 0.019 to 0.113 ± 0.022 ml/min/g after verapamil infusion (NS) and to 0.149 ± 0.024 ml/min/g after nitroglycerin (p < 0.01). Uncorrected MBF in the border zone increased significantly after infusion of both verapamil (0.469 ± 0.085 ml/min/g, p < 0.01) and nitroglycerin (0.398 ± 0.056, p < 0.05). When corrections were made for interdigitating normal tissue in the border zone, only the MBF after nitroglycerin was significantly increased. Thus, nitroglycerin significantly increased the collateral blood flow to ischemic tissue in the central ischemic and border zones, but verapamil did not.

THE MECHANISM by which verapamil and other calcium-channel blocking agents are effective in the treatment of patients with angina pectoris is not clear. These drugs decrease myocardial oxygen demand by decreasing blood pressure and also increase myocardial blood flow. Whether they can increase collateral blood flow to ischemic myocardium is uncertain.

Accordingly, we designed this study to observe and compare the direct effects of verapamil and nitroglycerin (NTG) on collateral blood flow in dogs when these drugs were administered by intracoronary infusion while coronary perfusion pressure was held constant.

Methods

Preparation

Twelve dogs were anesthetized with i.v. pentobarbital (30 mg/kg), intubated with an endotracheal tube and ventilated with 100% oxygen, delivered by a pressure-cycled ventilator (Bird, Mark 7). After a left thoracotomy, the left main coronary artery and left anterior descending coronary artery (LAD) distal to the first diagonal branch were dissected free. After administration of 10,000 U of heparin, the left main coronary artery was cannulated with a Gregg cannula inserted through the left subclavian artery and perfused from the left carotid artery (fig. 1). The LAD was separately cannulated and also perfused from this carotid line, but proximal to the left main cannula. A polyethylene cannula (0.03-inch i.d.,) was inserted into the proximal LAD at the site of the LAD cannulation, and the pressure in the left main system was measured. The pressure in the left main system was maintained constant by feeding the mean pressure signal from this cannula’s transducer to a servo amplifier (Harvard Apparatus Co., Model 990) and peristaltic pump (Harvard Apparatus Co., Model 1200 series) on the carotid perfusion line. Left ventricular pressure and dP/dt were monitored with a stiff polyvinyl cannula (0.067-inch i.d.) inserted into the left ventricle through the left atrial appendage. A mixing chamber, with a magnetic stirrer to ensure adequate mixing of injected microspheres, and an electromagnetic flowmeter were inserted into the carotid perfusion line.

Procedure

Regional myocardial blood flow (MBF) was measured using radioactive microspheres, 7–10 μ in diameter, labeled with 141Ce, 51Cr, 125I and 85Sr (3M Company).1 Approximately 5 × 108 microspheres were injected into the perfusion line proximal to the mixing chamber. The reference blood sample for calculation of absolute blood flow was withdrawn distally from the perfusion line at a rate of 4.2 ml/min.

The first bolus of microspheres was injected into the perfusion line distal to the LAD cannula. With normal carotid perfusion of both the LAD and the left main coronary, this set of the microspheres was selectively distributed to the remaining left ventricular myocardium, which was not perfused by the distal LAD.2 Thus, during the subsequent LAD occlusion the fraction of tissue in the ischemic region “contaminated” by flows from the normally perfused region could be calculated.

The LAD perfusion line was occluded, and thus the LAD region was perfused only by collateral blood flow. The servopump was then activated and the left main coronary artery perfused at constant pressure irrespective of coronary blood flow or systemic pressure. After 20 minutes of LAD occlusion, the collateral blood flow was measured by the microsphere technique and by retrograde bleeding from the LAD perfusion line into a graduated cylinder over 1–2 minutes, with the open end of the line being held at the level of the left ventricle.

Solutions of nitroglycerin (60 μg/ml) and verapamil
Figure 1. The experimental preparation. Normally perfused tissue was labeled with microspheres before clamping the perfusion line to the left anterior descending coronary artery by permitting normal left anterior descending perfusion with unlabeled blood. The pressure in the left main coronary artery was kept constant by feeding the signal from the proximal coronary pressure transducer to the servo amplifier and peristaltic pump.

(100 μg/ml) were infused into the perfusion line with a syringe pump (Harvard Apparatus), and total coronary blood flow was monitored. The infusion rates were increased in steps with the objective of increasing coronary flow monitored on the electromagnetic flow meter by approximately 50%. The infusion rates were then kept constant for 5 minutes and the blood flow measurements were made. Although the infusion rates were intended to increase coronary flow by 50%, the actual increases in monitored flow were 55 ± 10% for six dogs that received 14 μg verapamil/min, and 73 ± 10% for the six dogs receiving 36 μg verapamil/min, 30 ± 6% for six dogs receiving 19 μg NTG/min and 46 ± 12% for six dogs receiving 47 μg NTG/min. Although the objective of a 50% increase in monitored coronary blood flow was not regularly achieved, the results from all dogs were combined. NTG was infused in six dogs, followed by a washout period of 60 minutes before infusion of verapamil. In the other six dogs, the order of drug infusion was reversed.

The dogs were killed and the left main coronary artery and LAD were infused at equal pressures (approximately 100 mm Hg) with red and blue Monastral pigment dyes (Dupont) mixed with a gelatin solution. The hearts were fixed in 3.7% formalin solution and subsequently sectioned in 1-cm-thick layers perpendicular to the axis between the aortic valve and left ventricular apex. The entire LAD region was excised from each ring. This included a central ischemic region and a border ischemic region approximately 3-4 mm wide. The samples from the central ischemic zone were carefully dissected to exclude any color pigment injected into the left main coronary artery, whereas the border zone was excised from the periphery of the LAD region to include all the remaining color pigment infused into the LAD. Three samples from nons ischemic regions were also removed. All samples were divided into inner and outer halves and each was subdivided into specimens of approximately 1–2 g, and weighed. The reference blood, tissue samples and blood specimens collected during retrograde bleeding in four dogs were subjected to gamma spectrometry (Searle Analytic Model 1085 equipped with Nuclear Data Model ND60 pulse-height analyzer). The count rates were corrected for background and crossover counts (Wang Laboratories 2200 computer). Standard indicator dilution formulas were used to calculate MBF/unit weight tissue.3

The fraction of tissue from the ischemic and border regions “contaminated” by normally perfused interdigitating myocardium was calculated. Thus, MBFs in the “uncontaminated” or corrected ischemic tissue were calculated as follows: Let MBF in normal tissue during normal zone labeling be A ml/min/g and MBF in the sample tissue be B ml/min/g. Thus, the fraction of normally perfused tissue in the sample = B/A, and the fraction of unlabeled tissue in the sample = 1 - (B/A). Let the MBF during an intervention be C ml/min/g in the normal tissue, measured MBF in the sample tissue be D ml/min/g and corrected MBF in the sample be F ml/min/g. Thus, in the sample tissue, measured flow = normal zone flow + corrected flow.

\[
D = C \frac{B}{A} + F \left[1 - \frac{B}{A}\right]
\]

\[
F = \frac{D - C \frac{B}{A}}{1 - \frac{B}{A}}
\]

In these calculations we have assumed that flow in the remote normal tissue is the same as that in the normal tissue adjacent to the ischemic zone.

Statistics

The results of hemodynamics and blood flows during control ischemia, during infusion of verapamil and nitroglycerin were compared by one-way analysis of variance and the Newman-Keuls procedure. Results were regarded as significant when \( p < 0.05 \). Results are expressed as mean ± SEM.

Results

The mean pressure in the proximal coronary artery during control ischemia was 83 ± 4 mm Hg and was kept constant by the servopump throughout the experiment. The average infusion rate of intracoronary verapamil was 25 μg/min and NTG 33 μg/min during the measurement of pressures and blood flows.

The results of the hemodynamics during control ischemia, intracoronary verapamil and NTG infusions are shown in Table 1. There was no significant change in heart rate and peripheral coronary pressure in the occluded LAD during infusion of either drug. Verapamil infusion resulted in a small elevation in left ventricular end-diastolic pressure (LVEDP) and NTG in-
fusion a small reduction of LVEDP, although neither differed significantly from control ischemia. Both verapamil and NTG reduced left ventricular systolic pressure, but only the reduction by NTG was significant. Contractility, as measured by maximum positive dP/dt, was reduced by verapamil, but not by NTG.

The mean weight of ischemic myocardium determined from the initial microsphere injection was 33.8 ± 2.5 g. This included 29.4 ± 2.4 g of central ischemic tissue and 4.6 ± 0.6 g of border zone tissue. The myocardial specimens from the central ischemic zone were calculated as having 2.5 ± 0.3% normally perfused tissue, while those from the border zone 26.8 ± 4.3%. The mean left ventricular weight was 130.3 ± 5.2 g.

The MBFs from normally perfused tissue, central ischemic zone and border zone for the 12 dogs are shown in table 2. The infusion of each drug resulted in a significant increase in flow in the normally perfused zone. LAD occlusion resulted in marked reduction in MBF in the central ischemic zone. When these MBF measurements were corrected for the relatively small fraction of normal tissue in the specimens from the central ischemic zone the statistical significance of the results was not altered. NTG infusion caused a significantly greater increase in collateral blood flow to the ischemic myocardium than that produced by verapamil. The small increase in collateral blood flow after verapamil infusion was not significant. NTG infusion resulted in a significant increase in retrograde blood flow from the occluded LAD, whereas verapamil infusion did not (table 1). Both verapamil and NTG infusion resulted in an increase in uncorrected MBF in the border zone. When these flows were corrected for interdigitating normal tissue, only NTG caused a significant increase in blood flow to the ischemic region of the border zone.

To exclude the possibility that the calculated MBFs in the ischemic region were falsely reduced by loss of microspheres during retrograde blood flow collections, samples of these blood specimens were subjected to gamma spectrometry. The activity of these specimens was not different from background.

**Table 1. Hemodynamics During Verapamil and Nitroglycerin Infusion**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Verapamil</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>142 ± 6</td>
<td>139 ± 6</td>
<td>142 ± 6</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.3 ± 0.7</td>
<td>5.5 ± 1.0</td>
<td>3.4 ± 0.6†</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>97.0 ± 4.0</td>
<td>90.0 ± 4.9</td>
<td>87.2 ± 4.0*</td>
</tr>
<tr>
<td>PCP (mm Hg)</td>
<td>22.0 ± 1.6</td>
<td>21.7 ± 1.4</td>
<td>21.2 ± 1.4</td>
</tr>
<tr>
<td>dP/dt (mm Hg/sec)</td>
<td>1483 ± 104</td>
<td>1208 ± 87*</td>
<td>1440 ± 27</td>
</tr>
<tr>
<td>Retrograde blood flow (ml/min)</td>
<td>3.9 ± 0.8</td>
<td>3.8 ± 0.8</td>
<td>5.1 ± 1.1‡</td>
</tr>
</tbody>
</table>

* p < 0.05 vs control ischemia.
† p < 0.05 verapamil vs NTG.
§ p < 0.01 NTG vs verapamil/control ischemia.

**Table 2. Regional Myocardial Blood Flows**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Verapamil</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardial</td>
<td>0.794 ± 0.096</td>
<td>1.328 ± 0.229*</td>
<td>0.929 ± 0.161</td>
</tr>
<tr>
<td>Epicardial</td>
<td>0.931 ± 0.135</td>
<td>1.603 ± 0.227*</td>
<td>1.228 ± 0.151‡</td>
</tr>
<tr>
<td>Transmural</td>
<td>0.868 ± 0.113</td>
<td>1.374 ± 0.225*</td>
<td>1.171 ± 0.150‡</td>
</tr>
<tr>
<td>Ischemic zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardial</td>
<td>0.076 ± 0.014</td>
<td>0.077 ± 0.013</td>
<td>0.113 ± 0.016†</td>
</tr>
<tr>
<td>Epicardial</td>
<td>0.136 ± 0.026</td>
<td>0.145 ± 0.030</td>
<td>0.189 ± 0.034‡</td>
</tr>
<tr>
<td>Transmural</td>
<td>0.101 ± 0.019</td>
<td>0.113 ± 0.022</td>
<td>0.149 ± 0.024‡</td>
</tr>
<tr>
<td>Border zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unorrected</td>
<td>0.297 ± 0.047</td>
<td>0.469 ± 0.085*</td>
<td>0.398 ± 0.056‡</td>
</tr>
<tr>
<td>Corrected</td>
<td>0.165 ± 0.041</td>
<td>0.235 ± 0.062</td>
<td>0.268 ± 0.0438</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
* p < 0.01, verapamil vs control.
† p < 0.01, NTG vs control ischemia and NTG vs verapamil.
‡ p < 0.05 NTG vs control.
§ p < 0.05 NTG vs control.

**Discussion**

The objective of these experiments was to compare the direct effects of verapamil and NTG on coronary collateral blood flow where coronary perfusion pressure remained constant and the systemic effects of these drugs were minimal. Both drugs were given in doses that increased the MBF in the normal zone; however, only NTG significantly increased the collateral blood flow in the central ischemic zone as well as the collateral blood flow corrected for overlapping normal tissue in the border zone. The increase in flow in these regions after verapamil infusion was not significant.

The coronary perfusion pressure was kept constant throughout each experiment. However, there were small yet significant effects on the LVEDP and left ventricular systolic pressure during the 5-minute intra-coronary infusion of the drugs. The greater reduction in systolic pressure following NTG infusion was attributed to a decrease in both preload and afterload. The reduction in contractility, as reflected by a decrease in maximum dP/dt, was considered a direct myocardial-depressant effect of verapamil.

Our method of sectioning the hearts, which separated a peripheral 4 mm of ischemic tissue, resulted in a central ischemic zone of approximately 90% and a border zone of 10% of the myocardium at risk. Labeling the normal zone with microspheres enabled us to verify that the samples removed from the central ischemic zone indeed contained minimal interdigitating normal tissue, in contrast to those samples from the border zone.

In the central ischemic zone, MBFs during control ischemia were lower than generally found. This can be attributed to the relatively low mean perfusion pressure and the large area at risk. Another possible explanation for the low central ischemic zone flows is that microspheres could have been washed backward out of the capillaries during retrograde bleeding. This would imply that there were significant intercapillary collaterals. The absence of any significant number of micro-
spheres in blood collected during retrograde bleeding is evidence against loss of microspheres by this route and suggestive evidence against the existence of these collaterals.

NTG infusion resulted in a marked increase in collateral blood flow in the central ischemic region whereas the increase after verapamil infusion was not significant. Although we did not study dose-response curves, verapamil was administered in two doses, both of which caused significant reduction in small vessel resistance in the normally perfused myocardium and thus presumably in sufficiently large doses not to have have missed an effect on collateral blood flow. On the other hand, this greater increase in flow in the normally perfused vessels could diminish the stem pressure or pressure head from which the collateral vessels originate ("coronary steal"). To exclude the possibility that a marked reduction in stem pressure may have offset any significant reduction in collateral resistance, we separately analyzed the flow results from six dogs with the smallest increase in MBF in the normal zone after verapamil infusion and from the six dogs receiving the lower doses of verapamil. The mean increase in normal zone MBF of 29.9% in the former six dogs and 54.5% in the latter and no significant increase in collateral blood flow to the central ischemic zone was observed. However, we cannot completely exclude the possibility that smaller doses of verapamil increased the MBF to the central ischemic zone.

Because the collateral flow and peripheral coronary pressure did not change after verapamil, presumably a small reduction had occurred in the resistance of the collateral and possibly the epicardial vessels, thus avoiding an coronary steal. This is in contrast to NTG, which presumably produced a greater reduction in the collateral and epicardial vessel resistance and resulted in an increase in collateral flow. We previously reported a coronary steal after an intracoronary bolus of NTG administered under special circumstances. In this dog preparation, the entire subendocardium of the left ventricle had been made ischemic by reduction of flow in the left main coronary artery. The regional flow measurements were subsequently made during the peak reduction in small vessel resistance and before significant increases in large and penetrating vessel conductance had occurred.

The misleading conclusions of observing MBF in the border region without correcting for interdigitating normal tissue is evident by comparing the effects of the two coronary vasodilator drugs on this region before and after these corrections were made. The apparent marked increase in MBF in this region with verapamil was not significant when these corrections were made. In contrast, the uncorrected flows in the border zone after NTG infusion became significant after correction.

The corrected MBFs in the border regions should be interpreted with caution. During control ischemia, the calculated MBF in the border region was higher than that in the central ischemic zone. This calculation of MBF in the border zone depends upon the assumption that flow in the interdigitating normal tissue in this region is identical to that in the more distant normal myocardium. This assumption may not be correct. The normal tissue in the border zone may have a greater MBF because of its proximity to vasodilatory metabolites produced by ischemic tissue. In addition, tissue at the interface of normally contracting and dysfunctional myocardium may be subjected to greater wall stress. A 35% increase in MBF in the normally perfused fraction of the border zone can account for the difference in the corrected MBFs in the central ischemic and border ischemic zones. However, it could be attributed to a greater collateral blood flow (presumably by intramyocardial channels) to this region than to the central ischemic zone. We doubt whether this is the case because of the presence of discrete lateral border zone with end-capillary loops previously described in our laboratory. The technique of labeling the normal zone with microspheres is consequently useful in verifying tissues in which there is minimal contamination with interdigitating normal tissue. Thus, tissue samples representative of the central ischemic zone can be obtained that do not require correction for calculation of MBFs.

Our finding that verapamil did not increase collateral blood flow was not surprising, for we previously observed that it did not increase large vessel conductance and significantly decreased small vessel resistance. This is in contrast to NTG, which has been shown to increase large and also collateral vessel conductance and collateral blood flow. Our observation that verapamil did not significantly increase collateral blood flow to either the central ischemic or border zones is similar to that of Weintraub et al., who administered systemic nifedipine but without control of systemic blood pressure. Using methods that did not precisely define the region at risk, some authors have reported that verapamil increased the collateral blood flow in this region, whereas others have observed no change. Retrograde blood flow measurements have shown that verapamil increases the collateral flow whereas, after embolizing the microcirculation of the region at risk, others have failed to demonstrate a reduction in collateral resistance.

In conclusion, our experiments on collateral blood flow during acute ischemia in dogs have shown that intracoronary NTG, increased collateral blood flow, but verapamil did not. Thus, if our results can be extrapolated to patients, we suggest that mechanisms other than direct augmentation of collateral blood flow are responsible for verapamil's effectiveness in relieving effort-induced angina pectoris.

Acknowledgment

We gratefully acknowledge the skilful technical assistance of Herbert Parker and Walter Leon and the secretarial assistance of Marilyn Sasso.

References

2. Hirzel HO, Sonnenblick EH, Kirk ES: Absence of a lateral border zone of intermediate creatine phosphokinase depletion surrounding
Comparison of Rest and Exercise Radionuclide Angiography and Exercise Treadmill Testing for Diagnosis of Anatomically Extensive Coronary Artery Disease

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SUMMARY The accuracy of rest and exercise radionuclide angiography (RNA) and exercise treadmill testing (ETT) for diagnosis of three-vessel or left main coronary artery disease (extensive CAD) was determined in 544 patients. ETT and RNA sensitivities were similar (88% vs 92%, NS), but ETT was more specific than RNA (46% vs 34%, p < 0.01). The prevalence of extensive CAD in patients with a positive treadmill (41%) increased only 3% when the RNA was also positive. However, in the 292 patients with a negative or indeterminate ETT, a positive RNA increased this prevalence from 16% to 23%, while a negative RNA decreased this prevalence to 5%. These results support the initial use of ETT followed by RNA if the treadmill is negative or indeterminate for diagnosis in a population with a high prevalence of extensive CAD. This approach separates patients into subgroups with a high or low probability of extensive CAD.

SURVIVAL in patients with coronary artery disease (CAD) has been related to the number of diseased coronary vessels and to the presence of significant left main coronary artery stenosis. The identification of patients with a high probability of three-vessel or left main disease is an important goal of noninvasive testing. Exercise-induced left ventricular functional abnormalities appear to result from ischemia and can be used to diagnose CAD in patients with chest pain. Moreover, the magnitude of abnormality of left ventricular function during exercise appears to be related to the anatomic extent of coronary artery disease. The purpose of the present study is to document the relative diagnostic accuracy of radionuclide angiography (RNA) and exercise treadmill testing (ETT) in evaluating patients for the presence of three-vessel or left main CAD.

Methods

Study Population

Between January 1, 1977, and June 30, 1981, 3005 patients underwent cardiac catheterization and coronary arteriography for suspected coronary artery disease at Duke University Medical Center. Bruce multistage exercise treadmill tests were performed in 1495
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