Influence of the Extent of the Zone at Risk on the Effectiveness of Drugs in Reducing Infarct Size

LAIR G.T. RIBEIRO, M.D., WAI-MAN CHEUNG, M.S., AND PETER R. MAROKO, M.D.

SUMMARY The goal of this study was to examine whether the effectiveness of a drug in protecting ischemic myocardium depends on the size of the hypoperfused zone (the area at risk) measured immediately after coronary artery occlusion (CAO). Methoxy-verapamil (D600), a potent calcium antagonist, was used to test this hypothesis. In 68 dogs, 1 minute after CAO, 8 mCi of technetium-99m-labeled albumin microspheres were injected into the left atrium for later assessment of the hypoperfused zone by autoradiography. Eighteen dogs were treated with D600 (0.8 mg/kg as a bolus 15 minutes after CAO and 0.2 mg/kg/hour as a continuous infusion for 6 hours). After 6 hours, the hearts were excised and the left ventricles cut into 3-mm-thick slices and stained with triphenyltetrazolium chloride. The extent of myocardial damage was measured by planimetry of the unstained areas. Thereafter, the same slices were autoradiographed and the extent of the hypoperfused zones measured by planimetry of the “cold spots.”

Both the treated and control dogs were classified according to the amount of the left ventricle that was hypoperfused: small (<25%), medium (25–30%), and large (>30%). In control dogs with small, medium and large hypoperfused zones, the percentages of the hypoperfused zone that evolved to infarction were 95.9 ± 3.5% (mean ± SEM), 90.8 ± 3.5%, and 93.1 ± 2.6%, respectively; in the D600-treated dogs, 31.9 ± 8.3%, 53.8 ± 3.0%, and 61.3 ± 9.2%, respectively. Thus, the dogs with the smallest areas at risk had the most extensive reduction in damage (67%); the effectiveness of treatment was intermediate in those with medium areas at risk (41%) and treatment had the least effect in those with the largest area at risk (34%). Thus, the size of the area at risk, determined in vivo immediately after CAO, is an important factor in determining the effectiveness of a drug in reducing myocardial damage.

NUMEROUS INTERVENTIONS are effective in reducing the extent of myocardial damage after experimental coronary artery occlusion. However, it is not known whether, besides the beneficial properties of the intervention, the degree of effectiveness depends upon the initial size of the area at risk of infarction, i.e., the hypoperfused zone (HZ). The goal of this investigation was to determine whether methoxy-verapamil (D600), a potent calcium antagonist, reduces myocardial damage to the same extent in areas of hypoperfusion of different magnitudes. The area of hypoperfusion was determined by a recently developed technique that has the advantage of assessing the extent of the HZ immediately after coronary artery occlusion, in vivo, permitting evaluation of the extent of the HZ before intervention and under the actual conditions of collateral blood flow.

Methods

Sixty-eight mongrel dogs that weighed 17–22 kg were anesthetized with sodium thiamylal, endotracheally intubated, and ventilated with room air using a Harvard respirator. ECGs (lead aVF) and systemic arterial pressures through a polyethylene cannula in the left carotid artery (Statham P23Db pressure transducer) were recorded continuously throughout the experiments (Gould Instruments). The chest was opened in the fifth left intercostal space and the heart was suspended in a pericardial cradle. A polyethylene catheter was placed in the left atrium through its appendage to inject radiolabeled microspheres. The left anterior descending coronary artery was dissected free from the adjacent tissue and occluded permanently with a silk suture.

To assess the zone of hypoperfusion, 2 × 10⁶ highly radioactive (8 mCi) albumin microspheres, 20 μ in diameter (3M Company), were injected into the left atrium 1 minute after coronary artery occlusion. Eighteen minutes after occlusion, the dogs were randomized into two groups, 50 to a control group and 18 to a D600-treated group. In the latter, D600 was administered intravenously: 0.8 mg/kg as a bolus, followed by a continuous infusion of 0.2 mg/kg/hour until 6 hours after coronary artery occlusion.

Six hours after coronary artery occlusion, the dogs were killed and the left ventricle (LV) was dissected free from all other structures (i.e., the free wall of the right ventricle, atria, aorta and valves). The LV was frozen at −70°C for 30 minutes and was then cut into 20–25 3-mm-thick slices from apex to base. The slices were incubated in a 1% solution of triphenyltetrazolium chloride (TTC, Sigma Chemicals) for 10 minutes at 37°C. The normal tissue stained dark red and the damaged tissue pale yellow. A transparent plastic sheet was placed over the slices, and their contours and those of the yellow and red areas were traced onto the plastic. The total areas of the slices and the areas of damaged myocardium were calculated by planimetry (Apple II computer). Thereafter, the extent of the HZ was determined on the same slices. The slices were placed on a sheet of high-speed x-ray film (Cronex 4, E.I. DuPont) and image contrast was obtained with sensitive, medium-contrast enhancing screens. The film was exposed for 13 hours and developed (X-omat automatic processor). To identify the border, the same slices were exposed to a “soft” x-ray (25kVp–100 mA) and superimposed on the autoradiographs. The HZs (cold spots) were white, and the perfused areas (hot spots) were black (fig. 1). The contours of these images were transferred to a plastic overlay, and the

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total areas of the slices and the HZs were calculated by planimetry (Apple II computer).

The following measurements were calculated: (1) The percentage of the LV that showed myocardial damage by TTC staining. This zone is called infarct size (IS) and is expressed as a percentage of the LV. (2) The percentage of the LV that was initially hypoperfused as determined by autoradiography. This zone, the HZ, is expressed as a percentage of LV. (3) The percentage of the HZ that evolved to infarction. This was calculated by dividing IS by HZ and multiplying by 100. This represents the percentage of the area that was at risk because of reduced blood flow that actually evolved to myocardial tissue injury.

The weights of the IS and HZ were also calculated ponderally, using the weight of the LV and multiplying it by the IS and HZ, respectively.

The dogs were then classified according to the extent of the HZ: those with initial small, medium and large HZs (< 25, 25–30% and > 30% of the LV). The percentage of the HZ that evolved to infarction was analyzed in each of the three classes both in the control and the D600-treated dogs. Thereafter, this index (IS/HZ × 100) was compared between the treated and controls of each class; thus, the effectiveness of treatment could be assessed by calculating the ratio IS/HZ × 100 for the treated dogs and dividing it by the same ratio for the corresponding class of control dogs:

\[
\left[1 - \frac{\text{IS treated}}{\text{HZ treated}} \div \frac{\text{IS control}}{\text{HZ control}} \right] \times 100
\]

The resulting number is the percent reduction of the extent of damage (as normalized by the zone at risk) resulting from treatment in each class. The percent of reduction in IS was examined also as a continuous function of the HZ.

Comparisons between values obtained in the same dogs were made using a paired t test, and between treated and control dogs by t test for group observations. Analysis of variance was used to compare values among different subgroups. The Fisher exact test was used for evaluation of mortality. The results are presented as mean ± SEM.

**Results**

The HZs created by coronary artery occlusions in both treated and control animals were similar in magnitude. The HZ 1 minute after coronary artery occlusion was 26.2 ± 1.3% of the LV in the controls and 26.7 ± 2.3% of the LV in the treated dogs, showing that occlusion resulted in comparable areas at risk. Heart rate and mean systemic arterial pressure were similar in both groups 15 minutes after coronary artery occlusion and just before administration of D600 in the treated group (table 1). Therefore, before treatment started, the two groups were similar by the size of area at risk and by the measured hemodynamic variables.

In the control group, mean systemic arterial pressure did not change, while heart rate increased 6 hours after occlusion, by an average of 21 beats/min. In contrast, in the D600-treated group, mean arterial pressure fell by an average of about 16 mm Hg and heart rate slightly decreased (NS). Thus, in the treated dogs, both mean arterial pressure and heart rate were significantly lower than in the control dogs (table 1).

The incidence of death from ventricular fibrillation from 15 minutes to 6 hours after coronary artery occlusion was 20% (10 of 50 dogs) in the control group and 0% in the D600-treated group \( (\chi^2 = 4.22, p < 0.05) \) (fig. 2).

IS, determined by planimetry of slices stained with TTC, as a percentage of the LV, was 24.4 ± 0.8% in
the control group, which corresponded to 22.6 ± 1.4 g. IS was 14.1 ± 2.2% of the LV, or 13.6 ± 1.8 g, in the D600-treated group (p < 0.001). When IS was evaluated as a percent of the HZ, it was 93.6 ± 1.8% in the control dogs and was 49% smaller (i.e., 47.8 ± 5.0%) in the D600-treated dogs (p < 0.001, fig. 3).

To analyze whether the effectiveness of D600 in reducing ultimate IS is dependent on the initial area of hypoperfusion, the dogs were classified into those with an HZ less than 25% of the LV (small), those with 25–30% of the LV hypoperfused (medium), and those with more than 30% of the LV hypoperfused (large). The average size of the HZs of all three classes of the D600-treated dogs were similar to those in their respective controls (table 2).

In the control group, the percentage of the area of hypoperfusion that evolved to infarction was similar in the three classes, 95.9 ± 3.5%, 90.8 ± 3.5% and 93.1 ± 2.6% for small, medium and large HZs, respectively. In the D600-treated group, the percentage of HZ that evolved to infarction was smaller in the D600-treated dogs than in their respective controls for each class. For small, medium and large infarcts it was 31.9 ± 8.3%, 53.8 ± 3.0%, and 61.3 ± 9.2%. All these IS/HZ values are significantly smaller than their respective controls (p < 0.001). The reduction in IS/HZ was 67% in dogs with small, 41% in those with medium and 34% in those with large HZs. The effectiveness of D600 was significantly greater with the initial smaller area at risk than in the other two classes (fig. 4). When the data were analyzed as a continuous function in which the extent of salvage of the myocardium was a function of the area at risk, a close inverse correlation was found (r = −0.71), which shows that the smaller the HZ, the larger the salvaged zone (fig. 5). Thus, the smaller the initial zone in jeopardy, the more effective the treatment.

Table 1. Effects of D600 on Heart Rate and Arterial Pressure After Experimental Coronary Artery Occlusion

<table>
<thead>
<tr>
<th>Time after occlusion</th>
<th>Control</th>
<th>D600</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min*</td>
<td>146 ± 6</td>
<td>132 ± 6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>100 ± 5</td>
<td>99 ± 5</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>147 ± 5</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>30 min</td>
<td>100 ± 4</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>167 ± 3</td>
<td>129 ± 3</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>100 ± 4</td>
<td>83 ± 6</td>
</tr>
</tbody>
</table>

*Before drug administration.

Abbreviations: HR = heart rate; MAP = mean systemic arterial pressure.

![Figure 2](http://circ.ahajournals.org/)

**Table 2.** Mortality rate in dogs between 15 minutes and 6 hours after coronary artery occlusion. Mortality is shown as the percent of dogs that died in each group. Numbers over the columns are the number of dogs that died and the total of dogs in each group.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** The percent of the hypoperfused zone that evolved to necrosis — comparison of the control and the D600-treated groups. Each dot represents one dog.

**Discussion**

Myocardial infarction is a dynamic process. The ultimate size of an infarction can be modified by inter-
TABLE 2. Effectiveness of D600 in Reducing Infarct Size — Importance of the Extent of the Hypoperfused Zone

<table>
<thead>
<tr>
<th>Extent of hypoperfused zone</th>
<th>Hypoperfused zone (%LV)</th>
<th>Infarct size/hypoperfused zone Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small (&lt; 25% LV)</td>
<td>C 17.9 ± 1.2 (14)*</td>
<td>95.9 ± 3.5 67%</td>
</tr>
<tr>
<td></td>
<td>T 17.3 ± 2.4 (7)</td>
<td>31.9 ± 8.3†</td>
</tr>
<tr>
<td>Medium (25–30% LV)</td>
<td>C 27.7 ± 0.5 (10)</td>
<td>90.8 ± 3.5 41%</td>
</tr>
<tr>
<td></td>
<td>T 27.6 ± 0.9 (5)</td>
<td>53.8 ± 3.0</td>
</tr>
<tr>
<td>Large (&gt; 30% LV)</td>
<td>C 34.6 ± 1.2 (12)</td>
<td>93.1 ± 2.6 34%</td>
</tr>
<tr>
<td></td>
<td>T 37.0 ± 1.5 (6)</td>
<td>61.3 ± 9.2*</td>
</tr>
</tbody>
</table>

Abbreviations: C = control; T = treated.
*Numbers in brackets indicate the number of dogs.
†p < 0.001 vs control.

Interventions early after coronary artery occlusion, making it possible either to decrease or to increase IS.1–5, 12–16 Many experimental studies have confirmed and amplified this concept.17–19 Some interventions have been used in clinical trials and, based on indirect measurements of myocardial damage, are applicable in patients with myocardial infarction.20–25 However, it has been extremely difficult to compare the relative effectiveness of different interventions or the effectiveness of one intervention under different circumstances, either because the techniques were not quantitative,3 or because, although the IS was measured precisely, it showed a wide range of variability.17, 18 IS, even when the coronary arteries are occluded at the same site, can vary markedly in consequence of different anatomic distribution of the coronary arteries and the variability in collateral blood flow. Thus, occlusion of the left anterior descending artery at similar sites may produce infarcts ranging from 50% of the LV to zero. With this variability in individual size of infarcts, the differences between groups of treated and control dogs are difficult to interpret. Several techniques to circumvent this problem have been suggested.26–28

Techniques for determining the area at risk have used dyes, either in vivo just before sacrifice,29–30 or after sacrifice using artificial perfusion pressures.31–34 Other postmortem techniques have also been used, such as stereoscopic coronary arteriography.35, 36 Autoradiography has been used to determine the area at risk in vivo, just before sacrifice.37 However, all these determinations were performed after the intervention was given and thus would not represent the original area at risk if the intervention interfered with the collateral circulation.38 A step further was the development of a technique to determine the area at risk before intervention with technetium-99m-labeled albumin microspheres.8 Later, this technique was improved by cutting the heart into 20–25 slices, rather than only seven, and using “soft x-ray” radiographs to enhance the visibility of the border of each slice. This technique made it possible to detect with enhanced sensitivity the effects of beneficial interventions that, using other methods of measurement, would not have been considered effective.9 Recently, we have used this technique that makes each dog its own control and overcomes the problem of variability in the distribution of the coronary arteries.8, 9 The HZ is measured 1 minute after coronary artery occlusion under the actual hemodynamic conditions of the dog, including the collateral blood flow. This is done by injecting radioactive microspheres into the left atrium 1 minute after coronary artery occlusion and later cutting the LV into 3-mm-thick slices and submitting them to autoradiography and “soft” x-rays. Using this technique, one can compare the extent of the area at risk in each dog to the extent of the area of necrosis. The time of determination of the area at risk is crucial because the intervention itself, if given before the time of determination of the HZ, may alter the collateral blood flow and thus change the extent of the area of hypoperfusion, which should no longer be considered area at risk. In untreated dogs, 94 ± 2% of the area at risk evolves to necrosis. Moreover, there is a highly significant corre-
lation between the area at risk and ultimate necrosis: 
IS (%LV) = 0.89 ± 0.89HZ (%LV), n = 36, r = 0.93. Thus, the area at risk accurately predicts the ex-
tent of IS.

Using this technique, we tested the hypothesis that the extent of the initial HZ influences the effectiveness of an intervention in reducing IS. D600 was selected because it is a very potent calcium antagonist, with properties similar to verapamil, which is very effective in reducing arrhythmias and myocardial dam-
age after experimental coronary artery occlusion. We did not examine how D600 protects ischemic myocardium. However, we can speculate, based on previous studies, that D600 may either block the in-
flux of calcium to the cell, which may play an important role in protecting the cell, or may favorably alter the oxygen supply/demand ratio in the ische-
mic myocardium due to possible vasodilatory action or due to the decrease in myocardial oxygen consump-
tion.

In this study, D600 reduced IS and prevented vent-
tricular fibrillation after coronary artery occlusion. This latter property may be due to an antiarrhythmic effect or may be indirectly due to protection of the ischemic myocardium.

To judge whether the initial size of the area at risk is an important factor in determining the effectiveness of an intervention in reducing IS, both the control and treated dogs were grouped according to the extent of their areas at risk. Those with small areas at risk, when treated, showed a reduction in myocardial injury of 67% compared with the untreated dogs of the same class, while in those with large areas at risk, the reduc-
tion in myocardial injury by treatment was only 34% when compared with the untreated dogs of the same class. The effectiveness of treatment in dogs with med-
ium zones of hypoperfusion was intermediate i.e., 41% reduction. Thus, in dogs with small areas of hypoperfusion, the benefit of treatment was nearly twice that in dogs with large zones of hypoperfusion. Also, when the extent of salvaged myocardium was analyzed as a continuous function, there was a close inverse correlation between it and the magnitude of the hypo-
perfused myocardium. This can be explained by easier penetration of the drug into smaller ischemic zones due to higher surface/volume ratio. The observation that small zones of hypoperfusion present more collateral-
als is also in accordance with this concept.

We conclude that the effectiveness of an interven-
tion depends on the size of the area at risk and that the smaller this area, the more effective the treatment. In studies in which the relative effectiveness of different interventions are examined, the areas at risk of infar-
tion should be of similar magnitude.

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