Effects of Beta-Adrenergic Blockade on the Natural Progression of Myocardial Infarct Size and Compensatory Hypertrophy

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Using contrast-enhanced computed tomography, the effects of beta-adrenergic blockade were assessed on experimentally produced myocardial infarcts in dogs evaluated serially over the course of approximately 1 month. Infarct size, initial perfusion defect (jeopardized segment) and noninfarcted muscle mass were studied in two groups of conditioned mongrel dogs. Group 1 (n = 11) served as the control group and Group 2 (n = 10) was pretreated with propranolol (2 mg/kg). Each animal in the propranolol-treated group was given identical amounts of the agent twice daily for 7 days after coronary occlusion. Both groups developed increases in the noninfarcted muscle mass of the left ventricle (compensatory hypertrophy). The mean increase averaged 19.8% over 30 days when the two groups were included together. Infarct size was smaller in the propranolol-treated group, and averaged 28% less (p < 0.05) than that of the control group 30 days after initial myocardial infarction.

Thus, pharmacologic interventions were shown by computed tomography to alter the size of an acute experimental myocardial infarct, particularly when examined over the time course of infarct healing. Moreover, compensatory hypertrophy occurred in both the control and propranolol-treated groups.

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Quantification of myocardial infarct size is of prognostic importance in patients with acute ischemic damage (1,2). There have been a number of attempts to examine infarct size during therapeutic interventions (3). To a large extent, limitation of techniques for estimating infarct size, lack of knowledge of the natural progression of infarct size in vivo and the difficulty of differentiating between acute and long-term modifications in infarct size have made interpretation of the utility of these intervention studies unclear.

Recent implementation of contrast-enhanced computed tomography has allowed an assessment of myocardial infarct size. The ability of tomography to detect myocardial infarction has been evident since the report of Adams et al. (4). Since then, several studies (5–8) have examined the ability of computed tomography to detect and quantitate the size of myocardial infarcts in ex vivo and in vivo experimental models and, although specific protocols have differed, the findings indicate that X-ray transmission and tomography is a relatively accurate method for measuring infarct size. Moreover, we have validated tomographic infarct sizing in acute, chronic and reperfused infarcts and found accurate data available from images derived from each group (8–10). In addition, we recently studied (10) dogs serially over the course of 1 month after occlusion of the left anterior descending coronary artery using contrast-enhanced computed tomography. Computed tomographic scans were obtained acutely (20 minutes after coronary occlusion) and then at multiple time periods until sacrifice approximately 1 month later. We found that infarct size increased at 4 days when compared with initial perfusion defect, then progressively decreased. The initial perfusion defect overestimated the eventual infarct size at 1 month by 33 ± 15%. Noninfarcted left ventricular muscle mass increased significantly (27 ± 7% greater at 1 month compared with day 0) over time, presumably representing compensatory left ventricular hypertrophy. The left ventricular muscle mass at necropsy correlated well (r = 0.94, p < 0.001) with the tomographic muscle mass just before sacrifice of the dog.

Thus, the purposes of the current study were to evaluate the effects of beta-adrenergic blockade on computed tomographic estimates of infarct size over time and to examine the associated changes in compensatory hypertrophy of the noninfarcted residual muscle.
Methods

Study 1

Experimental model. The experiments were initially conducted in 26 conditioned mongrel dogs weighing from 22 to 35 kg. Each animal was anesthetized with intravenous sodium pentobarbital (25 mg/kg body weight) before a left thoracotomy was performed. A hydraulic occluder was placed around the proximal left anterior descending coronary artery. The catheter was burrowed subcutaneously and externalized. An injection catheter was placed into the left atrium and also burrowed subcutaneously. The wound was aseptically closed and the dog allowed to recover. Control computed tomographic scans were obtained 4 to 7 days after surgery in all animals to ensure that none developed infarction during the preparation of the initial experimental model. In addition, enzymatic analysis during the first 24 and also burrowed subcutaneously. The wound was aseptically closed and the dog allowed to recover. Control computed tomographic scans were obtained 4 to 7 days after surgery in all animals to ensure that none developed infarction during the preparation of the initial experimental model. In addition, enzymatic analysis during the first 24 to 48 hours after the preparation confirmed the later scan data.

Three or more days after the control scans were obtained, each animal was premedicated with subcutaneous morphine sulfate (2.5 to 3.0 mg/kg) and intramuscular procainamide (150 mg). The dog was anesthetized with sodium pentobarbital (25 mg/kg) intravenously, paralyzed with succinylcholine (1.0 to 1.5 mg/kg) intravenously and ventilated with a Harvard respirator (14 to 16 breaths/min, tidal volume 12 to 15 cc/kg). The hydraulic occluder was inflated with the animal in the scanner. Scanning was performed immediately after coronary occlusion and at 3 to 4, 7 to 11 and 28 to 32 days after occlusion. After the final scan, the animal was killed and postmortem examination was performed.

The dogs were randomly separated into two groups: group 1 served as a control and group 2 was given 2 mg/kg of propranolol hydrochloride (Ayerst Laboratories) intravenously as the “enhanced defect,” which represented the area of myocardium on images obtained after the infusion of contrast medium. On the scans obtained on later days, a second region in addition to the area of low attenuation was defined as the “enhanced defect,” which represented the area of myocardium demarcated by the outer margin of a region of delayed contrast enhancement at the periphery of the infarct zone. On delayed scans, the periphery of the infarct has greater enhancement than the normal myocardium and center of the infarct (12). This region of delayed enhancement corresponds to the region of accumulation of technetium-99m pyrophosphate in hearts with infarction (12). We have demonstrated the superior precision of infarct estimates when the contrast-enhanced segment is included (13). In our experience, the best representation of this was on the ungated images obtained at 10 minutes after cessation of the infusion (20 minutes from the start of the contrast infusion).

Computed transmission tomographic analyses. All studies were performed on a Technicare 2020 whole-body scanner with an individual scan time of 2 seconds. The scanning technique was set at 120 kVp, 100 mA, 10 mm slice thickness and a 25 cm scanning circle. The scanner has 720 stationary detectors. All images were reconstructed utilizing the same reconstruction algorithm with a 512 x 512 matrix.

On the day of infarction, an intravenous infusion of meglumine sodium diatrizoate (76%) was started 20 minutes after coronary occlusion of the left anterior descending coronary artery (above the first diagonal branch) at a rate of 6 cc/min (lasting for 10 minutes, total volume = 60 ml) using a Harvard infusion pump. For orientation purposes, a set of (ungated) scans were obtained from the apex to the base of the left ventricle (perpendicular to the long axis of the ventricle) at 10 minutes into the infusion period. The contrast infusion was then stopped. Ten to 15 minutes after cessation of the contrast infusion, a series of ungated scans was again obtained from the apex to the base of the heart. On every scan day thereafter, the same protocol was followed. All scans were obtained during held inspiration. The scans taken during the infusion defect and the scans obtained on subsequent days were used to quantify the contrast enhancement of the infarct itself and to quantitate infarct size.

Postmortem examination. Immediately after the final scans, each animal was killed and the heart was removed and sectioned at 1 cm intervals from apex to base along the major axis of the left ventricle. The slices were incubated in a 1% solution of triphenyltetrazolium chloride (Sigma Chemical), in phosphate buffer for 10 minutes at 37°C (11). The endocardial and epicardial borders of the left ventricular wall and the visible area of infarction were traced onto clear film overlays. Analysis of these overlays is described later.

Data analysis. Infarct size measurement. Each set of images (apex to base) was printed on transparent film along with spatial calibration markers. In each animal, on the initial occlusion day scans, one region from each slice was defined as the “perfusion defect.” This consisted of an area of lower attenuation compared with the normally enhancing myocardium on images obtained after the infusion of contrast medium. On the scans obtained on later days, a second region in addition to the area of low attenuation was defined as the “enhanced defect,” which represented the area of myocardium demarcated by the outer margin of a region of delayed contrast enhancement at the periphery of the infarct zone. On delayed scans, the periphery of the infarct has greater enhancement than the normal myocardium and center of the infarct (12). This region of delayed enhancement corresponds to the region of accumulation of technetium-99m pyrophosphate in hearts with infarction (12). We have demonstrated the superior precision of infarct estimates when the contrast-enhanced segment is included (13). In our experience, the best representation of this was on the ungated images obtained at 10 minutes after cessation of the infusion (20 minutes from the start of the contrast infusion).

Volume of the jeopardized and infarcted regions of the left ventricle was determined by planimetry utilizing a Hewlett-Packard computer/digitizer (9825/9874A) system with manual tracing of the “perfusion defect” and “enhanced defects,” respectively. The computer then calculated the area contained within the closed loop. The ventricular slices were each assumed to be cylindrical. The volume of the scan-calculated size or perfusion defect was obtained by summing the volume of infarcted tissue (each slice was 1 cm) for each individual slice from the apex to the base of the heart. The postmortem overlays were similarly determined by planimetry and the same geometric assumptions were made. We found that infarct size assessed by computed
tomography correlated well with postmortem values for all of the animals initially entered into the study ($r = 0.91$) (SEE = 2.1 g).

The variability of this technique in our laboratory when the slices are retracted and redigitized is $\pm 3.5\%$ (8). Moreover, when each scan is retracted and analyzed by a second blinded observer, the coefficient of variation is within 5%. Similar tracing, digitizing and integrating of slice data were performed on the postmortem overlays.

**Left ventricular mass estimate.** The volume of left ventricular myocardial mass was also calculated from the ungated images and the postmortem overlays. This was accomplished by planimetry of the left ventricular myocardium from the apex to the level of the outflow tract as previously described (10). The total muscle mass derived from computed tomography correlated well with postmortem values in our study ($r = 0.91$, $n = 21$). Noninfarcted mass was derived by subtracting infarct mass from total mass. The infarct volume was then divided by the volume of left ventricular mass yielding the percent of myocardium that was infarcted. All myocardial measurements were converted to mass assuming a density of 1.05 g/cm$^3$.

In six additional dogs, the computed tomographic and thallium planar measures of myocardial perfusion were compared. The animals were anesthetized and coronary occlusion was performed in the scanner, and 30 minutes later, 1 to 1.3 mCi of thallium-201 was administered intravenously. In each animal, the same vessel (left anterior descending coronary artery) was occluded: 10 minutes after thallium-201 was administered, contrast medium was given as an infusion (identical to the technique in the present study) and cross-sectional scans of 1 cm in depth were obtained. The vertical axis of the scans was aligned with the long axis of the left ventricle. Images were obtained from the apex to the base of the heart. Once the computed tomographic scans were reconstructed, the animal was killed by administering intravenous potassium chloride. The heart was removed and sectioned (1 cm depth) along the long axis of the left ventricle (analogous to the image acquisition). Each postmortem section was imaged with a standard gamma camera peaked and windowed for thallium-201. Both the X-ray computed tomographic and radionuclide (ungated) images were printed on transparent film along with spatial calibration markers. In each animal, the perfusion defect was assessed by planimetry (region of reduced attenuation on computed tomography or reduced radionuclide emissions on thallium scintigrams). The volume of the computed tomographic and radionuclide data was obtained by summing the cross-sectional volumes (each slice was 1 cm and the geometry of each section was assumed to be cylindrical) from the apex to base of the heart. The perfusion data from both techniques correlated well ($r = 0.96$, $p < 0.01$).

The relation between the initial perfusion defect, analogous to an area of jeopardy, and the subsequent volume of calculated infarction was analyzed over time by the ratio:

$$\text{Infarct/jeopardy ratio} = \frac{\text{Volume of enhanced defect}}{\text{Volume of perfusion defect}}$$

This calculation was made for each sampling point during the study.

**Study 2**

In an additional 12 dogs, the identical surgical protocol was followed. Two groups were again identified with six dogs each in the control and propranolol-treated groups. However, in contrast to the first study, each animal was killed 4 days after occlusion of the left anterior descending coronary artery. Once again a perfusion zone was determined immediately after coronary occlusion. At postmortem examination, the infarct zone was easily visualized and five to six transmural samples of approximately 1 cc volume were taken from the normal and infarct segments. The heart was sectioned and planimetry performed for calculated infarct zone as a percent of total muscle mass as previously described (8-10,13,14).

The samples were weighed (Mettlars Beam Balance), gently dried for 7 days in a vacuum oven and then reweighed. Multiple weighings within the 7 day interval ensured a stable dry weight. The water content of the infarct tissue (wet weight - dry weight)/wet weight) was then normalized to the values derived from control segments of myocardium from the noninfarct zone.

**Statistics.** All data are given as group mean ± standard deviation. Repeated measures analysis of variance was used to evaluate all raw data over time (15). The correlations of the final computed tomographic infarct size and total left ventricular mass with the pathologic specimen were obtained with a least-squares fit using linear regression analysis (see Methods). Groups were compared at the same time period with standard analysis of variance.

**Results**

**Acute hemodynamic changes.** After coronary occlusion, propranolol treatment resulted in an initial increase in mean left atrial pressure (6.2 ± 1.3 from 2.6 ± 1.2 mm Hg, $p < 0.01$) when compared with values in group 1 (control group) (3.1 ± 1.0 mm Hg, $p < 0.05$) along with a significant reduction in heart rate (129 ± 24 from 112 ± 23 beats/min, $p < 0.01$ versus group 1, 132 ± 24 beats/min in the control group) and mean arterial pressure (102 ± 15 from 119 ± 15 mm Hg, $p < 0.05$ versus 117 ± 14 mm Hg in the control group). At each scanning sequence, the heart rate for each group was measured. The heart rate from
The propranolol-treated group was 10 ± 3 beats/min slower during the day 4 scan (p < 0.01) when compared with that in the control group. During all subsequent scans, the heart rates from each group were similar.

**Scan data.** The initial perfusion defects were similar in both groups, and each perfusion defect represented a consistent proportion of the total left ventricular mass (Fig. 1, Table 1). The serial changes in infarct size in each group are shown in Table 2, and presented as a percent of non-infarct mass and as a ratio with the initial perfusion defect. Statistically significant reductions in infarct size as a percent of left ventricular mass were noted at 4, 9 and 30 days. The "infarct/jeopardy" ratio (infarct/perfusion defect) was progressively smaller when comparing the control group with the propranolol-treated group at day 4. This disparity altered as time passed, with the propranolol-treated group demonstrating the smallest infarct/jeopardy ratio on days 28 to 32 when compared with the control group (mean reduction of 29%, p < 0.01 versus control group).

When the second group of 4 day infarct animals was evaluated, the animals treated with propranolol (group 2) demonstrated similar bulk water content when compared with control animals (group 1) (Table 3).

**Compensatory hypertrophy.** Both groups developed compensatory hypertrophy by 30 days after initial infarction (Table 4). Finally, the correlation of postmortem and scan infarct size estimates was excellent (r = 0.91, SEE = 2.6 g). The postmortem (9.7 ± 4.1 g) and scan infarct (10.1 ± 3.9) estimates did not differ.

**Discussion**

Because few studies directed at evaluating the change in myocardial infarct size are available (10) and the reduction of infarct size is an important goal in the clinical management of acute ischemic injury (7,18), this study was devised to examine the effects of a pharmacologic agent (propranolol) on the natural progress of infarct size and compensatory hypertrophy. Ungated, contrast-enhanced computed tomography was the technique utilized to study infarct size and muscle mass over time. Both measurements have been repeatedly validated in experimental canine infarcts in this and other laboratories (4–10).

**Beta-adrenergic blockade in infarction.** Beta-adrenergic blocking agents have been proposed as possible agents for infarct size limitation because they have negative inotropic and chronotropic effects (10), reduce myocardial oxygen consumption (18) and alter myocardial perfusion characteristics (19). Intravenous propranolol (as administered chronically in this study for 7 days) is known to reduce cardiac output and systemic pressure as well as contractile performance (18,20). Both practolol and propranolol have been shown to reduce ST segment elevation and myocardial loss of creatine kinase (21,22); they have also been shown to reduce infarct size by some (23,24), but not all (25) investigators. Nonetheless, many studies (3) have demonstrated a decrease in long-term morbidity and mortality after the administration of beta-adrenergic blocking agents in patients with myocardial infarction. The relation to long-term control of ischemia, perfusion and arrhythmias may contribute to these findings. However, in the current study, we
found significant reductions in infarct size resulting from the administration of propranolol. It is possible that administered in a more continuous or sustained fashion (rather than twice daily in a brief 20 minute infusion), beta-adrenergic blocking agents can indeed be shown to limit infarct size even further. In the current study, the effects of general anesthesia may have minimized the beta-adrenergic blocking effects of the drug. It is possible that the membrane-stabilizing effects of the drug contributed to myocardial salvage (particularly at the doses used in this study).

Infarction and edema. From the data derived from study 2, it appeared that the reduction in infarct size (or infarct jeopardy zone) 4 days after coronary occlusion after propranolol administration was not related to a reduction in the water content in the infarct segment. These data suggest that edema control was not an important component in infarct size with beta-adrenergic blockade. The natural progression of infarct size shown in this and a previous study (10) may help to create a reference frame for assessing the usefulness of therapeutic interventions designed to modify infarct size. We found a significant increase in infarct size 4 days after coronary ligation predominantly due to edema formation. In a study of 4 and 28 days old infarcts, Reimer and Jennings (26) noted that early enlargement (4 days) of infarct volume was secondary to edema formation, hemorrhage and cellular infiltration. We also observed that the volume of the infarct progressively diminished in size after 4 days, with stabilization occurring around 3 to 4 weeks after coronary ligation. Moreover, the amount of bulk water in the infarct increases for the first several days after myocardial infarction, after which time water content decreases (and fibrous tissue increases) (27). Carlsson et al. (28) found a similar pattern of infarct shrinkage at 3 to 4 weeks.

Clinical implications. We conclude that computed tomography is an accurate technique for measuring and study-

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### Table 4. Analysis of Compensatory Hypertrophy of Noninfarcted Muscle

<table>
<thead>
<tr>
<th></th>
<th>0 Days</th>
<th>4 Days</th>
<th>9 Days</th>
<th>30 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Initial Total Muscle Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>78.6 ± 12.3</td>
<td>80.6 ± 13.3</td>
<td>90.2 ± 16.1*</td>
<td>95.9 ± 18.0†</td>
</tr>
<tr>
<td>Group 2</td>
<td>79.6 ± 13.6</td>
<td>82.5 ± 18.6</td>
<td>89.0 ± 15.5*</td>
<td>93.7 ± 17.1†</td>
</tr>
<tr>
<td>% Δ vs. Initial Total Muscle Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>2.5 ± 5.1</td>
<td>14.8 ± 12.9</td>
<td>22.0 ± 14.1</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>3.6 ± 3.6</td>
<td>11.8 ± 15.3</td>
<td>17.7 ± 17.4</td>
<td></td>
</tr>
</tbody>
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*p < 0.01 vs. day 0; †p < 0.005 vs. day 0.
sizing changes in myocardial infarct size and left ventricular muscle mass over time in experimental animals. In particular, its ability to determine the effects of drugs on infarct size and compensatory increases in left ventricular mass may prove clinically important in defining high and low risk patient groups. This may be particularly true when cine computed tomography is available. In the present canine study, the utility of administering propranolol to reduce infarct size has been examined. Although caution is advised in the clinical application of these results, it is apparent that studies designed to show the long-term reduction of infarct size must take into account the natural and somewhat variable regression of quantitative infarct size.

References