Evaluation of Methods for the Quantification of Experimental Myocardial Infarction

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SUMMARY Several invasive and noninvasive techniques used in determining the size of experimental myocardial infarction were evaluated after acute ligation of the left anterior descending (LAD) coronary artery in ten dogs. Systemic blood pressure, left ventricular end-diastolic pressure (LVEDP), and heart rate did not change significantly for up to 24 hours after coronary occlusion. Left ventricular wall motion abnormalities were detected by left ventriculography in the distribution of the LAD but these changes did not correlate well with the infarct weight determined at autopsy.

THE DEVELOPMENT OF A TECHNIQUE which rapidly and accurately determines the presence and extent of myocardial injury after coronary artery occlusion has been an elusive, but important goal. Immediate and long term disability in patients with myocardial infarction depends in large part on the degree of myocardial damage. Mortality from cardiogenic shock, ventricular dysrhythmia, and left ventricular dysfunction following acute myocardial infarction appears to be directly related to the extent of myocardial necrosis. Furthermore, the selection of patients for coronary artery bypass surgery might be influenced by the detection of recent myocardial necrosis if the latter could be reliably measured.

In recent years, it has been demonstrated that infarct size can be modified by pharmacologic and hemodynamic interventions in acute experimental preparations and perhaps in man as well. An accurate means of determining infarct size could provide information that would be potentially useful for evaluating the effectiveness of interventions used to protect ischemic myocardium. Serial measurements of infarct size might also provide information about the natural history of infarcts and allow correlations between infarct size and ultimate left ventricular functional impairment.

Acute myocardial injury has been assessed in the past in the experimental animal by various techniques including ventriculography, epicardial electrocardiography, radionuclide imaging, histochemical staining, histologic...
examination\textsuperscript{13} and serum creatinine phosphokinase (CPK) enzyme and isoenzyme analysis.\textsuperscript{13, 14} This study was undertaken to evaluate the accuracy of several invasive and noninvasive techniques in determining the size of experimental myocardial infarction.

Each technique was quantitated independently by a different observer who was unaware of the results from the other measurements.

Methods

Experiments were carried out in ten adult mongrel dogs of both sexes weighing 15 to 25 kg. After induction of anesthesia with sodium pentobarbital (25 mg/kg), a cuffed endotracheal tube was inserted and respiration maintained by a Harvard respirator. Under direct fluoroscopic control, a no. 6FR Lehman catheter was advanced retrograde from the femoral artery into the ascending aorta, and when needed, placed in the left ventricle to obtain pressure recordings. Central aortic pressure and left ventricular end-diastolic pressure (LVEDP) were recorded with Statham P23 Ia transducers on a multichannel Electronics for Medicine DR8 oscillographic recorder before and at regular intervals after coronary artery occlusion. Lead II on the standard electrocardiogram was also monitored continuously.

A median sternotomy was performed and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was permanently occluded with a ligature at the junction of its proximal and middle thirds. A 36 unit cloth grid was moistened and molded to the shape of the epicardial surface of the heart and oriented along the interventricular groove in a manner that was constant from dog to dog. Epicardial electrograms were obtained 15 min after coronary occlusion by the application of a hand-held electrode to each of the 36 sites (fig. 1). This time was chosen because previous investigators\textsuperscript{15, 16} have demonstrated a close relationship between epicardial ST-segment elevation 15 min after coronary occlusion and the degree of myocardial necrosis 24 hours later documented by tissue CPK depletion and histological examination.

Unipolar epicardial electrocardiography was performed at one-tenth sensitivity (1 mm = 1 mV) and a permanent record made on a Hewlett Packard 7712 recorder. The number of sites with ST-segment elevation equal to or greater than 2 mm (NST) as well as the sum of ST-segment elevations on all 36 epicardial sites (Σ ST) were determined in each experiment. In the interpretation of ST segment changes, the midpoint of a sloping ST segment was chosen as the point of reference and any beat with a QRS duration exceeding 0.065 sec was excluded from analysis.

Left ventriculography was performed in the RAO position after the manual injection of 10cc Renografin-76. The ventriculograms were recorded on 16 mm film with a single plane cine fluorographic unit and images representing consecutive end systole and end diastole were later traced. Each heart tracing was divided into anterior (A) and posterior (B) portions by a line drawn from the midpoint of the aortic valve to the left ventricular apex and the respective systolic and diastolic areas of A and B determined planimetrically before and one hour following coronary ligation. By comparing the change in areas from diastole to systole prior to and following coronary occlusion, an index of regional (anterior vs. posterior) ventricular function was estimated and expressed as percent change in area A or B with coronary ligation.

The chest was closed 30 min after coronary occlusion with chest tube drainage connected to an underwater seal. One hour after occlusion, 20mCi of \textsuperscript{99m}Tc-glucoshephotonate (TcGH) (supplied by New England Nuclear) was injected intravenously and images of the myocardial uptake of the radiopharmaceutical were obtained on a Picker Dyna Camera 2C gamma scintillation camera with a low energy, high resolution collimator. Imaging was performed immediately prior to sacrifice, either 8 hours (5 dogs) or 24 hours (5 dogs) after occlusion (7 or 23 hours after injection).

After sacrifice, the hearts were removed, imaged in an approximately anterior-posterior position and sectioned horizontally at 1 cm intervals in an axis perpendicular to the interventricular groove. The slices were then imaged and immediately thereafter incubated in buffered nitroblue tetrazolium (NBT), a yellow dehydrogenase which turns blue in the presence of dehydrogenases and thereby stains the exposed surface of normal myocardium blue. Infarcted myocardium which is depleted of dehydrogenases remains unstained.\textsuperscript{17} Thus, the borders of an infarct were clearly delineated in each slice.

Kodachromes of the stained slices were projected on tracing paper and the perimeters of the infarct and normal myocardium were traced. The fraction of each slice occupied by the infarct was determined by planimetry and this fraction times the weight of the slice gave the weight of the infarct in that slice. The total weight in grams of the infarct was calculated as the sum of the weights in each slice (fig. 2). Scintigraphic infarct size in cm\textsuperscript{2} was determined by planimetry of the projected cross-sectional area of radionuclide uptake on the right anterior oblique (RAO) projection and corrected to life size.

Myocardial tissue samples were selected from infarcted,
borderline and normal regions of the heart, stained with hematoxylin and eosin and periodic acid Schiff reagent, and examined for histological evidence of the presence and extent of necrosis.

All data are expressed in the text, tables, and figures as mean ± SEM. Statistical comparisons were carried out using the Student’s t-test and regression analyses were performed using the least squares method and deriving correlation coefficients.

**Results**

For purposes of analysis, all ten animals will be considered together since hemodynamic measurements and mean infarct size did not vary significantly whether the dogs were sacrificed at 8 or 24 hours after coronary occlusion. Mean blood pressure, heart rate, and LVEDP did not change significantly at any time after myocardial infarction; blood pressure decreased from 108 ± 5 to 103 ± 6 mm Hg, heart rate increased from 130 ± 7 to 142 ± 9 beats/min and LVEDP increased from 5.1 ± 1.5 to 6 ± 2 mm Hg.

Left ventriculography demonstrated a decrease in anterior wall contractility from 44 ± 5 to 22 ± 5% (P < 0.025) whereas that of the posterior wall of the left ventricle remained essentially unchanged from 30 ± 4 to 27 ± 4% (fig. 3). The development of a ventriculographically demonstrated large area of paradoxic movements was common in these canine myocardial infarcts and was demonstrated by left ventriculography in six of ten cases. The percent decrease in relative anterior wall contractility one hour after coronary occlusion did not, however, correlate closely with infarct weight determined at autopsy (r = 0.51).

A significant positive correlation was found between NST and infarct weight at autopsy (r = 0.93) as shown in figure 4. A similar correlation between Σ ST and infarct weight was also found: r = 0.91 (x = 2.44y + 16.65). A wide range of infarct sizes from less than 1 to 28 g was observed despite the

**FIGURE 2.** Quantitation of myocardial infarct size determined by nitroblue tetrazolium method (NBT).

**FIGURE 3.** Changes in relative contractility as demonstrated by ventriculography of area A (anterior left ventricle) and area B (posterior left ventricle) prior to and one hour after coronary occlusion.
Scintigrams of the excised hearts showed intense TcGH uptake over the antero-apical region of the left ventricle. The heart slices measuring about one centimeter in thickness showed the increased uptake to be concentrated in the anterior wall corresponding to the anatomic extent of the infarct as documented by NBT myocardial staining (fig. 8). That the nitroblue tetrazolium technique accurately delineated areas of recent myocardial infarction was confirmed by histological study of the same tissue (fig. 9). The correlation between in vivo scintigraphic infarct size and infarct weight at autopsy was excellent ($r = 0.94$) as noted in figure 10.

**Discussion**

Recent studies have shown that various pharmacologic and mechanical interventions including intra-aortic balloon counterpulsation, corticosteroids, hyaluronidase and nitroglycerin can reduce the size of experimentally produced myocardial infarcts under certain conditions. Whether these interventions will be as effective in man is unclear. In order to evaluate these and other therapeutic interventions in patients, however, objective and reliable methods, preferably although not necessarily noninvasive, which accurately determine infarct size and differentiate areas of ischemia from areas of necrosis, must be developed.

The selection of patients for surgery might also be influenced by the development of methods which identify myocardial necrosis. The high operative mortality rate for surgical intervention in the management of acute infarcts might be reduced if accurate preoperative assessment of the

**FIGURE 4.** Correlation between the number of sites on epicardial grid with $\geq 2$ mm positive deflection (NST) and the myocardial infarct weight in grams determined by nitroblue tetrazolium staining technique (NBT): $r = 0.93$ ($x = 0.52y + 4.74$).

Myocardial imaging in these surgically instrumented animals with median sternotomies was possible in nine of ten experiments. A large hemithorax obscured the entire left chest in a single case, precluding accurate TcGH scanning. Examples of scintigrams of small (5.1g) and large (17.9g) infarcts are shown in figures 5 and 6. The TcGH uptake is shown clearly just above the liver. The surgical incision labeled "scar" is only faintly visualized. Epicardial ST-segment mapping and in vivo scintigraphic infarct size determined from the RAO projection were shown to correlate closely ($r = 0.85$) as demonstrated in figure 7.

**FIGURE 5.** Myocardial scintigram with $^{99m}$Tc-glucoheptonate (TcGH) taken 24 hours post coronary occlusion. Infarct weight at autopsy determined by nitroblue tetrazolium technique (NBT) was 5.1 grams.

**FIGURE 6.** Myocardial scintigram with $^{99m}$Tc-glucoheptonate (TcGH) taken 8 hours post coronary occlusion. Infarct weight at autopsy determined by nitroblue tetrazolium technique (NBT) was 17.9 grams.
presence and extent of myocardial necrosis were possible. Because surgical intervention in patients with acute myocardial infarcts above 40% of left ventricular mass is associated with an extremely high operative mortality,29 identification of this group might lead to the selection of other therapeutic approaches.

Epicardial ST-segment mapping 15 minutes after coronary artery occlusion has previously been shown to correlate with myocardial CPK depletion and histological extent of infarction 24 hours after coronary occlusion.16, 18 Our data confirm the fact that epicardial ST mapping performed in this fashion is a valid predictor of infarct size. Recent work,24 however, suggests that ST-segment changes may not be specific for myocardial ischemia and that artificial elevations may occur related to electrolyte flux and other local myocardial changes. Although the genesis of ST-segment elevations is multifactorial and controversial, this study demonstrates that acute ST-segment elevations can be reliably correlated with autopsy-determined myocardial necrosis at either 8 or 24 hours after coronary occlusion.

Epicardial mapping appears to reflect injury to myocardial cells rather than areas of hypoperfusion, and this characteristic allows an objective means of comparing different postmyocardial infarction groups with variable coronary artery anatomy and of determining their response to interventions aimed at reducing infarct size. Predicted size on the basis of 15 minute epicardial ST segment mapping can be compared with measured infarct size after intervention and correlations made to determine the efficacy of a given therapeutic regimen. Precordial ST-segment mapping, although shown to correlate well with epicardial mapping in the experimental animal,28 has not been uniformly successful in man,28 and further investigation is needed to determine if this modality may be useful as an indicator of myocardial damage in clinical studies.

Our finding that infarct size was similar whether measured 8 or 24 hours after coronary ligation suggests that when blood pressure and heart rate remain at pre-infarction levels most of the myocardial necrosis that might occur during the evolution of an acute infarct is already determined by 8 hours after coronary occlusion. This supports the concept that the early hours after myocardial infarction are the critical ones during which interventions to decrease infarct size should be applied.7, 28

Ventriculography has been used as a means of evaluating ventricular function and localizing specific areas of abnormal wall motion.29 This study was not designed to determine whether an area of dyskinesia represents nonreversible myocardial damage but there is strong evidence that in fact paradoxical wall motion does not necessarily mean that cell death has occurred.29 It is, therefore, not surprising that the correlation in this study between abnormal ventricular wall motion 1 hour after coronary occlusion and infarct weight determined at autopsy was relatively poor. Nonetheless, localization of ventricular wall motion abnormalities can be accurately detected by ventriculography and in conjunction with other hemodynamic data can aid in determining the degree of functional myocardial impairment.

Myocardial infarcts as early as 8 hours old are frequently visible grossly but the borders are not clearly defined. The nitroblue tetrazolium technique, however, provides an ac-

Figure 7. Correlation between the number of sites on epicardial grid with ≥2 mm positive deflection (NST) and infarct size as determined by myocardial scintigraphy with $^{99m}$Tc-glucophone tonate ($^{99m}$TcGH): $r = 0.85$ ($x = 0.89y + 2.44$).

Figure 8. Comparison of myocardial necrosis detected by $^{99m}$Tc-glucophone tonate scintigraphy ($^{99m}$TcGH) and nitroblue tetrazolium myocardial staining technique (NBT): $r = 0.94$ ($x = 0.72y + 3.16$).

Figure 9. Correlation of nitroblue tetrazolium (NBT) determined myocardial injury and histologic evidence for myocardial damage. On the left, a section of unstained (infarcted) myocardium is shown in brackets. On the right, light microscopic evaluation after $H + E$ stain under $80 \times$ magnification reveals marked karyolysis, hypereosinophilic clumping of cytoplasm, and polymorphonuclear leukocyte infiltration.
In conclusion, acute myocardial infarct scintigraphy provides a direct noninvasive assessment of the presence and extent of acute myocardial necrosis. The radiopharmaceutical agent used most frequently in coronary care units for infarct detection, $^{99m}$Tc-pyrophosphate, has been reported to result in better quality images than TcGH, but uptake in bone structures can obscure the limits of the infarct with this agent and most commonly 12 to 24 hours must pass after coronary occlusion before reliable scans can be obtained. Imaging with TcGH has strong potential for providing accurate information about the size of an infarct very early in its course, when therapy can be most effective in preserving myocardium. TcGH scintigraphic infarct size correlates well with both predicted myocardial damage (epicardial ST-segment mapping) and determined infarct weight at autopsy (NBT myocardial histochemical staining).

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HYPERTENSION AND MYOCARDIAL ISCHEMIA/Loeb et al. 41

Effects of Pharmacologically-Induced Hypertension on Myocardial Ischemia and Coronary Hemodynamics in Patients with Fixed Coronary Obstruction

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SUMMARY Twenty patients with fixed coronary artery obstruction were studied during rapid atrial pacing and methoxamine infusion. During pacing to heart rates of 142 ± 4 (mean ± SEM) beats per minute coronary sinus flow increased from 108 ± 8 to 187 ± 15 cc/min and myocardial oxygen consumption increased by 80 ± 11%. During methoxamine infusion that raised arterial systolic pressure to 196 ± 5 mm Hg, similar increases in coronary sinus flow (to 197 ± 13 cc/min) and myocardial oxygen consumption (77 ± 12%) occurred. Chest pain and ischemic ST segment changes developed in 17 and 14 patients respectively during atrial pacing, an incidence significantly greater (P < 0.05) than during infusion of methoxamine (6 and 3 patients). Myocardial lactate extraction which averaged 26 ± 4% during control was decreased to 10 ± 8% during pacing and to 24 ± 7% during methoxamine; the difference between decreases was not significant. The data show that at similar increases in myocardial oxygen consumption stress of increased heart rate results in more myocardial ischemia than stress of increased afterload.

ELEVATION OF ARTERIAL PRESSURE is frequently considered an important mechanism leading to myocardial ischemia in patients with coronary artery disease. For this reason acute reduction in arterial pressure to reduce myocardial oxygen needs has been recommended for hypertensive patients with unstable angina or acute myocardial infarction. However, since an elevated arterial pressure also may improve perfusion to areas supplied by partially obstructed vessels, it is difficult to predict the overall effects of altering arterial pressure on myocardial oxygen supply and demand. We have examined the effects of hypertension induced by methoxamine infusion on coronary hemodynamics, myocardial metabolism, and clinical signs of myocardial ischemia in 20 patients with significant coronary artery disease. For comparison, the effects of rapid...
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