The importance of defining left ventricular area at risk in vivo during acute myocardial infarction: an experimental evaluation with myocardial contrast two-dimensional echocardiography

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ABSTRACT Because the left ventricular "area at risk" is the most important determinant of ultimate infarct size, it would be useful to know the size of the area at risk during acute myocardial infarction to make therapeutic decisions. We therefore performed a series of experiments in four groups of dogs. In group I dogs (n = 15) we attempted to determine whether current methods of assessing left ventricular function during acute myocardial infarction reflect the true size of the area at risk. At each of two to five sequential stages, a more proximal coronary occlusion was performed to produce a larger area at risk until cardiovascular collapse occurred. At each stage, the area at risk (measured by myocardial contrast echocardiography), hemodynamic variables, and left ventricular ejection fraction (LVEF) were measured. Hemodynamic variables became abnormal when the area at risk was large (25% to 40% of the left ventricle), whereas LVEF became abnormal when the area at risk was of moderate size (18%). When cardiac output and LVEF were normalized to baseline values, a close inverse relationship was noted between these variables and area at risk. In contrast, there was a poor relationship between normalized mean arterial pressure and area at risk (r = .42). In group II dogs (n = 9) the area at risk was measured serially over 6 hr after coronary occlusion. The size of the area at risk remained unchanged regardless of the transmural extent of the ultimate infarct. The circumferential endocardial extent of the area at risk closely predicted the circumferential endocardial extent of the infarct at 6 hr in eight of nine dogs that developed an infarct. Group III dogs (n = 7) underwent the same protocol as group II dogs, but the duration of occlusion was 3 hr. The circumferential endocardial extent of the area at risk closely predicted the circumferential endocardial extent of the infarct. Group IV dogs (n = 5) underwent subtotal coronary occlusion. Although regional wall motion abnormalities were noted in this group, no area at risk could be defined. We conclude that (1) although a close inverse relationship is noted between normalized cardiac output and area at risk, the absolute values for cardiac output and other hemodynamic variables become abnormal only when the area at risk is large (25% to 40%); (2) measurement of LVEF may provide a better assessment of the size of the area at risk than hemodynamic variables; (3) the circumferential endocardial extent of the area at risk closely predicts that of the ultimate infarct at both 3 and 6 hr after occlusion; (4) although abnormal wall motion is always present in the region of the area at risk, the converse is not true; and (5) a direct method of measuring area at risk such as myocardial contrast echocardiography would have several advantages in this era of interventional cardiology.


POSTMORTEM STUDIES have demonstrated that the size of the perfusion territory of an occluded coronary artery (area at risk) is the most important determinant of the ultimate infarct size. After coronary occlusion, the infarct begins in the endocardium and progresses transmurally over time. Apart from the size of the area at risk, therefore, the duration of coronary occlusion and the status of the collateral circulation to the ischemic zone also determine the ultimate infarct size. It therefore seems logical to provide either thrombolytic therapy or coronary angioplasty for patients with acute myocardial infarction as soon after the onset of symptoms as possible. However, rather
than provide such therapies with their accompanying complications to every patient with acute myocardial infarction, it may be more advisable to offer such therapy to those who may be at risk for developing larger infarcts and who therefore have a worse prognosis.

Until recently, there has been no readily available method of defining area at risk in vivo. Methods used previously to define area at risk in the experimental laboratory have been postmortem techniques such as technetium autoradiography, coronary arteriography, and injection of colored dyes into the coronary circulation. In the clinical situation, indirect measures of the size of the area at risk such as hemodynamic monitoring and radionuclide angiography to measure left ventricular ejection fraction (LVEF) have been used in the setting of acute myocardial infarction. However, no studies have been performed to determine whether these variables truly reflect the size of the area at risk and therefore whether they can be used to direct therapeutic decisions in the early hours of acute myocardial infarction.

The goals of this study, therefore, were: (1) to determine whether hemodynamic variables and LVEF reflect the size of the left ventricular area at risk during acute myocardial infarction; (2) to establish a relationship between left ventricular area at risk and left ventricular systolic function; (3) to examine whether the area at risk changes over time and to establish a relationship between area at risk and ultimate infarct size; and (4) to demonstrate that a direct estimation of the area at risk is required to differentiate abnormal wall motion occurring as a result of total coronary occlusion from that occurring during ischemia in the absence of total coronary occlusion. Area at risk was measured in vivo by myocardial contrast two-dimensional echocardiography (MCE), which is a new technique that has been demonstrated to define area at risk accurately in vivo. In addition, it has been found to be safe in both animals and humans and also has the potential for measuring regional myocardial blood flow.

Methods

Four groups of mongrel dogs were used for these experiments. In group I dogs (n = 23, weight 25.0 ± 30.0 kg), progressively larger areas of the myocardium were made ischemic during two to five sequential stages by occluding either the left anterior descending (LAD) or the left circumflex (LCx) coronary artery more proximally at each stage. MCE was performed at each stage to define the left ventricular area at risk and hemodynamic measurements were recorded. In 13 animals, LVEF was measured by radionuclide blood pool imaging. In group II dogs (n = 11, weight 24.0 ± 3.0 kg), either the LAD or LCx was occluded. MCE was performed serially over a 6 hr period and the animal was killed to measure infarct size. In group III dogs (n = 7, weight 26.0 ± 5.0 kg), the same procedure was performed except for a shorter occlusion period (3 hr). In group IV dogs (n = 5, weight 27.0 ± 3.0 kg), a subtotal coronary occlusion was performed and MCE was done serially over a 6 hr period. In group II, III, and IV dogs, mean arterial pressure was kept constant throughout the experiment.

Animal preparation. All dogs were anesthetized with 30 mg/kg iv sodium pentobarbital (Abbott Laboratories, North Chicago), intubated, and ventilated with a dual-phase control respirator pump (Model 607, Harvard Apparatus, South Natick, MA). Additional anesthesia was given as needed during the experiment. A median sternotomy was performed and the heart was suspended in a pericardial cradle. Catheters (No. 8F) were placed in the right and left femoral veins for intravenous administration of drugs and fluids as needed. A small proximal branch of the LAD was isolated and a 3.2 cm No. 22 F polyethylene catheter (Quick-Cath, Travenol Laboratories, Deerfield IL) was introduced in a retrograde manner into the lumen of the vessel, the tip of which was placed at the bifurcation of the left main coronary artery. This catheter was used to inject contrast medium into the left coronary system during MCE.

In group I dogs (figure 1), the sinoatrial node was mechanically crushed and the right atrium paced at a rate of 110 beats/min by an electrical stimulator (Grass Instruments, Quincy, MA). A No. 2F polyethylene catheter was placed in the ascending aorta via the right internal mammary artery for recording the central aortic pressure. A similar catheter was placed in the left atrium for recording the left atrial pressure. A No. 7F balloon-tipped thermolumination catheter (American Edwards Laboratory, Santa Anna, CA) was placed in the pulmonary artery to measure cardiac output. The proximal port of this catheter was used to measure the right atrial pressure. All these catheters were connected to a four-channel recorder (Model 2400S, Gould Instruments, Oxnard, CA) via fluid-filled pressure transducers (Model P231D, Gould Instruments). A No. 7F micromanometer-tipped catheter (Model Mikrotip PC-470, Millar Instruments, Houston) was introduced retrogradely into the left ventricle via the right femoral artery after calibration with a mercury manometer. This catheter and standard electrocardiographic leads were connected to another multichannel recorder (Irex Continual, Irex Medical Systems, Upper Saddle River, NJ) for the simultaneous recording of the left ventricular pressures and the electrocardiogram. Either the LAD or the LCx was dissected free from the surrounding tissue at four to five loca-
tions along its length so that occlusions of the artery at more proximal locations would render progressively larger areas of the myocardium ischemic. The sites of dissection were therefore chosen at the sites of major branches. Silk ties were loosely placed around the artery at these sites.

In group II, III, and IV dogs either the LAD or the LCx was isolated at its midportion and a tie was placed loosely around it. A No. 8F catheter was placed in the right femoral artery to monitor arterial pressure and was connected to a multichannel recorder (Model 4568C, Hewlett Packard Corp., Waltham, MA) via a fluid-filled transducer (Model 1280C, Hewlett Packard Corp.). Standard electrocardiographic leads were also placed on the animals and connected to the multichannel recorder.

**Hemodynamic measurements.** In group I dogs, all channels on the Gould 2800S recorder were calibrated at each stage of the experiment and all pressures monitored throughout the experiment at slow paper speed (0.5 mm/sec). Mean right and left atrial and phasic and mean aortic pressures were recorded for 20 beats at a paper speed of 25 mm/sec during each stage. Left ventricular pressures and the electrocardiogram were recorded simultaneously for 20 beats during each stage on the Irex Continual recorder at a paper speed of 200 mm/sec. An average thermodilution cardiac output was estimated at each stage of the experiment from three separate measurements. In group II, III, and IV dogs, the electrocardiogram and mean aortic pressure were recorded throughout the experiment at a paper speed of 0.5 mm/sec.

**MCE.** MCE was performed with a commercially available mechanical sector-scanning system with a 5 MHz transducer (Mark III, Advanced Technology Laboratories, Seattle, WA). Images were recorded on one-half inch videotape with a VHS recorder (either Model 8200 or Model 8950, Panasonic Corp. Japan). A saline bath acted as an acoustic interface between the heart and the transducer.

In group I dogs, the heart was imaged at baseline and at 15 min after each occlusion. Five short-axis levels (mitral valve, chordal, high and low papillary muscles, and apex) were recorded by manually moving the transducer from one level to the other over the anterior surface of the heart. Images were acquired at each short-axis level during the injection of 2 ml of contrast through the catheter placed in the left main coronary artery bifurcation. A hand-agitated mixture of equal amounts of saline and Renografin-76 (diatrizoate meglumine and diatrizoate sodium, 18.5 g/50 ml, E.R. Squibb and Sons, Princeton, NJ) was used as a contrast agent. This mixture contains microbubbles ranging in size from 2 to 25 μm (mean ± 1 SD 12 ± 7). These microbubbles cause transient left ventricular and systemic hemodynamic alterations without producing any pathologic effects in the myocardium. An off-line image analysis system (Microsonics, Easy View II, Microsonics Corp., Indianapolis) was used for analysis of the echo images. The video recordings were initially reviewed to selected optimal postcontrast cycles, which were then transferred to a video disc system (SVM 1010, Sony Corp., Japan) for making measurements. Area at risk and myocardial area were digitized for each level. To determine the total left ventricular area at risk, the areas at risk for all levels were added and expressed as a percentage of the total myocardial area from all levels as previously described. We have previously demonstrated a good interobserver and intraobserver correlation for measurement of area at risk. The interobserver correlation was 4 = .94 (p < .005) with an error of 0.42 cm² and the intraobserver correlation was r = .99 (p < .0001) with an error of 0.39 cm².

In group II, III, and IV dogs, the echocardiographic transducer was fixed at the midpapillary muscle level by means of a clamp affixed to the procedure table. In group II and IV dogs, MCE was performed 15 min and 2, 4, and 6 hr after occlusion. In group III dogs, MCE was performed at 15 min and 3 hr after coronary occlusion. Two milliliters of sonicated Renografin-76 was injected into the catheter placed in the left main coronary artery bifurcation. Sonication was performed with a commercially available sonicator system (Model W-375, Heat Systems Ultrasonics, Farmingdale, NY) at 20,000 cycles/sec for 30 sec at an energy output of 75 W. This method produces microbubbles with a mean size of 4 μm (range 1 to 7). We have demonstrated that 2 ml of sonicated Renografin-76 produces transient hemodynamic abnormalities in humans, which are less severe than those produced by 5 to 10 ml of unsonicated Renografin-76 used during routine coronary arteriography. Area at risk was determined with a calibrated off-line analysis system (Medical Image Processing System, Kontron Electronics, Eching, W. Germany). The endocardial circumferential extent of the area at risk and the entire area at risk were measured and expressed as a percentage of the endocardial circumference and myocardial area, respectively.

**Radionuclide angiography.** Radionuclide angiography was performed in the last 13 group I dogs for the estimation of LVEF at each stage of the experiment. Thirty minutes before the baseline study, 10 ml of blood was withdrawn from the animal and incubated for 15 min in vitro with 25 mCi of technetium-99m pertechnetate, after which the red cells were washed and suspended in a saline solution. We have previously demonstrated that this technique allows for optimal radionuclide labeling of red cells. The effective radionuclide dose injected after this procedure varied from 17 to 20 mCi. The red cell suspension was injected into the animal 20 min after injection of 3 ml of stannous pyrophosphate, and baseline imaging was begun 20 min thereafter. Imaging was initiated 20 min after each coronary occlusion. A mobile single-crystal gamma camera (Picker Dynano Corp., Solon, OH) with a general-purpose collimator was used for imaging the dogs in the left anterior oblique projection for optimal chamber separation. All data were acquired on 10 megabyte disks with a dedicated mobile computer (A², MDS Corp., Ann Arbor, MI) and an electrocardiographic gating device (Brattle Corp., Cambridge, MA). Data were acquired at 32 frames/cardiac cycle until 500,000 counts were collected for the end-diastolic frame.

LVEF was calculated by a fixed region of interest method. Background (mean counts/pixel) was calculated from a region five pixels lateral to the left ventricular blood pool in the end-diastolic frame and subtracted from each pixel in each frame. The computer then generated a time-activity curve from the 32 background-corrected frames. LVEF was calculated as the percent change in left ventricular counts between the end-diastolic and end-systolic frames. Figure 2 illustrates the end-diastolic and end-systolic frames obtained in an animal at baseline and at three different stages of occlusion of the LAD.

**Estimation of infarct size.** In group II, III, and IV dogs, infarct size was measured at the end of the experiment. A 3 inch long spinal needle was placed through the heart at the level of the echocardiographic transducer to identify the plane at which the infarct was to be measured. The heart was removed from the thorax, and the epicardial fat, great vessels, atria, and right ventricle were discarded. The heart was cut on either side of the needle, and a 1 cm slice of the left ventricle was obtained corresponding to the short-axis view during MCE. This slice was soaked in a solution of 1.3% 2,3,5-triphenyltetrazolium chloride (Sigma Chemical Co., St. Louis) in 0.2M Sorenson’s buffer (KH₂PO₄ and K₂HPO₄ in distilled water, pH 7.4) at 37°C for 20 min and then fixed in a 10% formalin solution. An image of each slice was obtained in the off-line analysis system, (Kontron Electronics) using a video camera with a resolution of 600 lines/field (66 series, Dage-MTI Corp., France).
FIGURE 2. End-diastolic and end-systolic frames during radionuclide angiography at baseline and three postocclusion stages involving the LAD. Progressive worsening of LVEF with increase in left ventricular dimensions is seen.

Michigan City, IN). The off-line analysis system was calibrated and the myocardial area of the slice measured. Infarct size was then expressed as a percentage of the myocardial area at that short-axis level. In addition, the endocardial extent of the infarct was expressed as a percentage of the entire endocardial circumference of the slice. Figure 3, A, illustrates a short-axis view of the heart obtained during MCE, showing an area at risk after LCx occlusion. Figure 3, B, illustrates an infarct in the same region obtained after staining the heart with 2,3,5-triphenyltetrazolium chloride.

Protocol. In group I dogs, the right atrial pressure was established at 5 to 10 mm Hg 30 min before baseline measurements. Ten milliliters of blood was withdrawn from the animal in a syringe containing 25 mCi of technetium pertechnetate, after which 3 ml of stannous pyrophosphate was injected into the animal. Twenty minutes later, the radiolabeled red cell suspension was injected into the animal. A bolus of lidocaine (1 mg/kg) was injected intravenously followed by a 1 mg/min continuous infusion. MCE was performed, followed by radionuclide angiography. During the latter, hemodynamic measurements were also performed and cardiac output measured. The first coronary occlusion was performed, and if the animal survived the occlusion all measurements were repeated 10 min after the occlusion. This sequence of events was repeated for each occlusion until the dog died of pump failure or refractory ventricular fibrillation.

In group II, III, and IV dogs, 1 mg/kg lidocaine was given intravenously followed by a 1 mg/min infusion. Either the LAD or LCx was occluded, and MCE was performed 15 min after occlusion. In group II and IV animals, MCE was also performed at 2, 4, and 6 hr after occlusion, and in group III animals it was performed at 3 hr after occlusion. The mean arterial pressure was kept constant at 90 to 100 mm Hg for the duration of the experiment with an infusion of phenylephrine hydrochloride (Winthrop-Breon Laboratories, New York). Group II and IV dogs were killed 6 hr after occlusion and infarct size was measured. In group III dogs, the infarct size was measured 3 hr after coronary occlusion.

Statistical analysis. All data are expressed as mean ± 1 SD. Comparison of data between different stages in group I dogs, or at different times in group II dogs, was done with MULTICOMPARE (RS/1, Bolt, Beranek, and Newman, Cambridge, MA). An interstage difference was considered significant at p < .01. MULTICOMPARE tests normality (Kolmogorov/Smirnov test) and homogeneity of variance (Levene’s test) in the population being studied and determines differences in means by the Newman-Keul’s multiple range test. Comparisons between normalized data (cardiac output, LVEF, and mean arterial pressure) and area at risk were performed by linear regression analysis (RS/1), and Fisher’s Z test was used to determine whether the slope of the correlation was different from unity. Similar analyses were performed for comparing infarct size and area at risk.

Results

We could obtain data beyond baseline in only 15 of the 23 group I dogs; the remaining eight died of ventricular fibrillation shortly after the first coronary occlusion. Of these 15 dogs, eight underwent LAD and seven underwent LCx occlusions. Two had intractable ventricular fibrillation after MCE and hemodynamic measurements in stage I but before cardiac output measurements could be made. Thirteen dogs survived the second occlusion, 12 survived the third occlusion, seven survived the fourth occlusion, and only two survived the fifth occlusion. The average duration of the experiment after the first occlusion was 2.5 hr. Although radionuclide angiography was performed in the last 13 dogs, only eight survived beyond baseline. Complete data could be obtained in nine of the 11 group II dogs; the rest died of ventricular fibrillation within the first 30 min of coronary occlusion. Five of these nine dogs underwent LAD and four underwent LCx occlusions. Data could be obtained in all seven of the group III dogs (four with LAD and three with LCx occlusions) and all five of the group IV dogs (two with LAD and three with LCx occlusions).
Relationship of hemodynamic data to left ventricular area at risk. Whereas MCE-defined area at risk was significantly different at each stage in group I dogs (figure 4), hemodynamic data were not. Thus, although cardiac output decreased progressively during each occlusion, the decrease became significant only when the area at risk was large (40% of the left ventricle) (figure 5, A). Similarly, although the left ventricular end-diastolic pressure rose during each occlusion, the increase became significant only when the area at risk was 25% (figure 5, B). In comparison, there was no significant difference in the left atrial pressure between different stages (figure 5, C). Similar results were obtained for mean right atrial pressure. The mean arterial pressure was maintained at near-normal levels until the area at risk was 40% (figure 5, D). Therefore, although hemodynamic data did become abnormal with progressive occlusions, they became significantly abnormal only when the area at risk was large.

Relationship between left ventricular systolic function and area at risk. Because heart rate may change in either direction after coronary occlusion and therefore make it difficult to define a relationship between systolic left ventricular function and area at risk, we paced the right
atrium of all dogs at a fixed rate after crushing the sinoatrial node. We examined three variables of left ventricular systolic function in this setting: cardiac output, mean arterial pressure, and LVEF. We have already described the relationship between area at risk and absolute cardiac output measurements and area at risk and absolute mean arterial pressure (see above). Figure 6 shows the relationship between area at risk and LVEF. LVEF becomes significantly abnormal when the area at risk is 18% and appears to be a more sensitive indicator of the size of the area at risk than the hemodynamic variables listed above. Figure 7 compares the size of the area at risk with cardiac output, mean arterial pressure, and LVEF, for which all values have been normalized to those at baseline. In this situation it can be appreciated that there is a close inverse relationship between normalized cardiac output (\(\text{CO}^*\)) and area at risk (AR) (figure 7, A), where \(\text{CO}^* = -1.3 \, \text{AR} + 99.0\) (\(n = 13\) dogs, \(r = .92, p < .001\)). The slope of the curve is not different from unity. Similarly, normalized LVEF (\(\text{LVEF}^*\)) also had a close inverse relationship to area at risk (figure 7, B), where \(\text{LVEF}^* = -1.0 \, \text{AR} + 93.0\) (\(n = 8\) dogs, \(r = .80, p < .001\)). In contrast, the relationship of normalized mean arterial pressure (\(\text{MAP}^*\)) to area at risk is poor (figure 7, C), where \(\text{MAP}^* = -0.5 \, \text{AR} + 101.5\) (\(n = 15\) dogs, \(r = .41, p < .001\)). Some animals demonstrated an increase
in the mean arterial pressure with areas at risk of 10% to 30%.

Relationship of area at risk defined at different intervals after coronary occlusion to ultimate infarct size. MCE-defined area at risk remained remarkably stable over time. Figure 8, A, illustrates that there is no significant change in the area at risk over a 6 hr period in group II dogs. Similar results were obtained over a 3 hr period in group III dogs. In addition, as can be seen in figure 3, A, area at risk is always transmural and the transmural extent of the area at risk also does not change over time. Figure 8, B, illustrates the relationship between area at risk and infarct size in the group II dogs. Eight of the nine developed infarcts at 6 hr and the one that did not had a 20% area at risk. However, another dog with an area at risk of similar size developed an infarct. The relationship between area at risk and infarct size (IS) is linear (IS = 1.0 AR − 10.5; r = .86, p < .003), with AR consistently overestimating infarct size. The mean area at risk is 31.9 ± 9.4%, whereas the mean infarct size is 22.5 ± 11.3% (p = .003). The mean infarct size/area at risk ratio is 0.67 ± 0.27. The circumferential endocardial extent of the area at risk, however, closely approximates the circumferential endocardial extent of the infarct in the animals that developed infarctions (figure 8, C) (y = 0.97x − 1.2; r = .96, p < .001). The mean circumferential endocardial extent of the area at risk is 33.6 ± 10.6%, while that of the infarct is 29.1 ± 14.6% (p = NS).

Figure 9, A, illustrates the relationship between area at risk and infarct size at 3 hr after coronary occlusion. The relationship is linear, but the slope of the relationship is less than that in figure 7, B (IS = 1.21 AR − 19.0; r = .98, p < .001). The mean area at risk is 24.4 ± 7.3% and the mean infarct size is 10.4 ± 8.9%. The infarct size/area at risk ratio is less than that in group II dogs (0.37 ± 0.24%; p = .03). One dog with a 15% area at risk did not develop an infarct, and two dogs with approximately 19% areas at risk developed infarcts involving about 5% of the left ventricle. However, as in group II dogs, the circumferential endocardial extent of the area at risk closely predicted the endocardial circumferential extent of the infarct (figure 9, B) (y = 1.1x − 2.4; r = .98, p < .001). The slope of this curve is not significantly different from that in figure 8, C. The mean circumferential extent of the area at risk is 23.4 ± 6.0, while that of the infarct is 20.1 ± 10.7 (p = NS).

Relationship of subtotal occlusion to area at risk. In all dogs in groups I, II, and III, wall motion abnormality was also noted in the region of the area at risk. In all those dogs, however, total coronary occlusion had been performed. To demonstrate that regional wall motion abnormality can occur without evidence of the area at risk, we performed subtotal coronary occlusion in five dogs for 6 hr. Regional wall motion abnormality was noted in all dogs, but area at risk could not be defined with MCE. At the end of 6 hr, three dogs had
FIGURE 8. A, Size of area at risk in group II dogs at various intervals following coronary occlusion. The area at risk appears to remain stable during the first 6 hr after occlusion. B, Relationship between area at risk and ultimate infarct size in nine group II dogs in which coronary occlusion was maintained for 6 hr. The relationship is linear, with area at risk consistently overestimating infarct size. C, Relationship between the circumferential endocardial extent of area at risk (measured at 15 min after coronary occlusion) and the circumferential endocardial extent of the ultimate infarct after 6 hr of coronary occlusion in the eight dogs that developed infarcts. The relationship is close.

FIGURE 9. A, Relationship between area at risk and ultimate infarct size in seven group III dogs in which coronary occlusion was maintained for 3 hr. The relationship is linear, with area at risk consistently overestimating infarct size. (The dotted lines represent the 95% confidence limits). B, Relationship between the circumferential endocardial extent of area at risk (measured at 15 min after coronary occlusion) and the circumferential endocardial extent of the ultimate infarct after 3 hr of coronary occlusion in the six dogs that developed infarcts. The relationship is close and is not different from that seen in group II dogs (figure 7, C). (The dotted lines represent the 95% confidence limits.)
no evidence of infarct, one dog demonstrated partial infarct of the posteromedial papillary muscle with no involvement of the rest of the myocardium, and one demonstrated partial infarct of the posteromedial papillary muscle along with minimal and barely detectable infarct in a small area of the left ventricular endocardium.

Discussion

Relationship between hemodynamic variables and area at risk. Our results indicate that although hemodynamic variables become abnormal after coronary occlusion, the degree of abnormality does not truly reflect the size of the area at risk. These values become significantly abnormal only when the area at risk is large (25% to 40% of the left ventricle). We paced the dogs in this study at a constant heart rate to exclude the influence of changes in heart rate on hemodynamic variables. It is possible that changes in heart rate caused by coronary occlusion (tachycardia or bradycardia) would have resulted in a more unpredictable relationship between these values and area at risk. In addition, our results demonstrate that instead of decreasing, the mean arterial pressure may increase with areas at risk of moderate size, probably secondary to catecholamine discharge after coronary occlusion. These data would therefore indicate that hemodynamic variables cannot be used during acute myocardial infarction to size the area at risk and make therapeutic decisions based on this information. These are important observations, since mean arterial pressure and other hemodynamic variables are routinely used to classify patients with acute myocardial infarction into various hemodynamic subsets.

Relationship between area at risk and left ventricular systolic function. In this study, we used cardiac output (at a fixed heart rate), mean arterial pressure, and LVEF as indicators of systolic function. Apart from mean arterial pressure, the other two variables had a close inverse relationship with area at risk when the values were normalized to those at baseline. Thus the larger the risk area, the lower the normalized cardiac output and normalized LVEF. Unfortunately, the relationship between normalized cardiac output and area at risk is not useful in the clinical situation for two obvious reasons. First, the baseline, preclosure cardiac output value is required to define this relationship. Knowing the preclosure baseline value is essential because of the large variability of baseline cardiac output in individuals. Second, the heart rate was held constant in our experiment. In most cases in the clinical situation, cardiac output may be maintained despite sizeable area at risk due to reflex tachycardia. On the other hand, cardiac output may be low despite an insignificant area at risk because of bradycardia. Low cardiac output in the absence of right ventricular infarction,27 bradycardia,28 or hypovolemia29 is usually noted with extensive myocardial necrosis.9, 29, 30

Compared with hemodynamic data (including cardiac output), LVEF values in our study became significantly abnormal with area at risk of moderate size (18%), suggesting the LVEF may be a more sensitive indicator of the size of the area at risk. Schneider et al.31 also found a close relationship between LVEF and area at risk. Therefore, although hyperkinesia of the uninvolved myocardium may compensate for the loss in systolic function, the compensation does not appear to be adequate when area at risk is equal to or greater than 18% of the left ventricular myocardium. Despite these encouraging results, however, LVEF measurements in the clinical setting have limitations. First, there is a wide variability in normal LVEF values.24 Thus a fall of 15% could represent the standard deviation of the measurement seen in normal subjects.24 In addition, a significant interobserver variability is seen in calculation of LVEF with computer-based, semiautomated techniques.24

Variability in MCE-defined area at risk over time. Our results indicate that MCE-defined area at risk remains constant over a 6 hr period. In addition, the area at risk always appears transmural during MCE. These data are in agreement with those of West et al.,32 who demonstrated that the size of AR remains stable over a 48 hour period following coronary occlusion in closed chest dogs. In contrast, Kemper et al.33 reported that the area at risk changes at 2 hr after occlusion from transmural to nontransmural in dogs that ultimately had nontransmural infarctions. In these dogs, contrast enhancement of the subepicardial region of the myocardium could be seen at 2 hr after coronary occlusion. These authors indicated that MCE could therefore be used at 2 hr after coronary occlusion to predict the transmural extent of the ultimate infarct. In contrast, although the transmural extents of the ultimate infarcts in our animals varied, MCE-defined area at risk remained transmural throughout the duration of the experiments. There could be two alternative explanations for the disparity between our results and those of Kemper et al. First, we used small microbubbles (mean size 4 μm) whereas they used large microbubbles (mean size 50 μm). Since the contrast effect of microbubbles is related to the sixth power of its diameter, larger bubbles will produce more contrast effect than smaller bubbles.34 Therefore, in a situation where
regional myocardial blood flow is reduced, only a few microbubbles may reach the ischemic bed; however, if they are large, they may result in some degree of myocardial opacification not noted with smaller bubbles. However, West et al.22 also used larger microbubbles and did not see this effect. Second, Kemper et al. used hydrogen peroxide to make microbubbles in vivo from blood-hydrogen peroxide reaction. It is possible that some of the hydrogen peroxide entered the ischemic bed via epicardial collaterals and reacted with blood after arriving there, resulting in the formation of large microbubbles capable of producing myocardial contrast enhancement.

Relationship between MCE-defined area at risk and ultimate infarct size. Our results indicate that the relationship between area at risk and infarct size is close and linear both at 3 and 6 hr after coronary occlusion. The slope of the relationship and the infarct size/area at risk ratio are significantly less at 3 hr compared with 6 hr after occlusion. These data imply that for areas at risk of the same size, infarct size is smaller at 3 than at 6 hr. These findings are in agreement with previously published data obtained by post-mortem methods of defining area at risk.1,4 Our 6 hr occlusion data are also similar to previous values obtained with MCE.15,35,36 One of our group II dogs with a 20% area at risk did not develop an infarct, whereas another with a similar area at risk did. Similarly, one of our group III dogs with a 15% area at risk did not develop an infarct, whereas two with approximately 19% areas at risk developed small infarcts (about 5%). These data are somewhat in agreement with those of Koyanagi et al.,7 who reported that infarcts do not develop in dogs with an area at risk of less than 18%. Minor differences between their data and ours can be explained by the differences in techniques and the variability in the collateral blood supply in dogs.

Of interest is the finding that although the relationship between area at risk and infarct size is dependent on the duration of coronary occlusion, the circumferential endocardial extent of the area at risk closely predicts the circumferential endocardial extent of the ultimate infarct regardless of the duration of occlusion. That the circumferential endocardial extent of the ultimate infarct is predetermined at the time of coronary occlusion is in agreement with earlier findings of Reimer et al.,4 who used postmortem methods of determining area at risk. These findings may have clinical significance because the circumferential endocardial extent of the area at risk and the duration of symptoms may be the two most important variables used to determine whether or not to provide interventional therapy during acute myocardial infarction.

Additional value of MCE over regional wall motion analysis. We have previously demonstrated that the relationship between area at risk and the extent of abnormal wall motion on two-dimensional echocardiography is dependent on both the method of assessing wall motion and the criterion used to determine abnormality.37 Thus, if a center of reference derived from only end-diastolic and end-systolic frames is used, less than 10% endocardial excursion seems to have the best relationship with area at risk; in contrast, dyskinesia or akinesia underestimate area at risk while less than 20% endocardial excursion overestimates area at risk. On the other hand, if the entire systolic contraction sequence is used and the center of reference is an average derived from the center of area from all frames between end-diastole and end-systole, the correlation between area at risk and extent of abnormal wall motion is good. Despite these findings, there are several disadvantages of using extent of abnormal wall motion as a precise indicator of the size of area at risk. First, wall motion is highly load dependent, and relatively hypoperfused areas may demonstrate normal to near-normal motion as a passive phenomenon when load is increased. Second, although quantitation of wall motion from the entire systolic contraction sequence minimizes errors in estimation of abnormal wall motion caused by temporal and spatial inhomogeneity of regional contraction and cardiac translation, it is tedious and time consuming and therefore is not clinically useful. Third, wall motion abnormalities will occur when antegrade coronary flow is low whether or not total coronary occlusion is present, as demonstrated in our group IV dogs. Because the initial optimal management of acute ischemic syndrome is dependent on whether or not total occlusion is present (thrombolytic therapy or angioplasty in the former5,6 and antianginal and/or anticoagulant therapy in the latter38,39), presence of abnormal wall motion alone may not be adequate to make a clinical decision.

Advantages of directly measuring area at risk in the clinical setting. From this study it is obvious that the direct estimation of the area at risk is optimal for the management of a patient with acute myocardial infarction. The presence or absence of area at risk and its size could help formulate rational therapeutic decisions. MCE is a recently described method of assessing area at risk in vivo12-15 and has been shown to be safe in both animals and humans.16,17 It also has the potential for measuring regional myocardial blood flow by means of washout.
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