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EXPERIMENTAL STUDIES

Contrast Echocardiography in Acute Myocardial Ischemia. II. The Effect of Site of Injection of Contrast Agent on the Estimation of Area at Risk for Necrosis After Coronary Occlusion

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Myocardial contrast echocardiography has been shown to accurately assess the area at risk for necrosis after acute coronary occlusion in the experimental model. The area at risk as determined by this method, however, has been defined in different ways depending on the model used. Some investigators have injected the contrast agent proximal to the site of coronary occlusion (left main coronary artery or aorta) and defined the area at risk as the segment of myocardium not showing a contrast effect (negative risk area). Others have injected the contrast agent directly into the occluded vessel and have defined the area at risk as that showing contrast enhancement (positive risk area).

To evaluate whether the areas at risk determined by these two techniques are identical, six open chest dogs were studied using both methods. The area at risk was slightly but significantly larger when the contrast agent was injected into the occluded vessel than when it was injected proximally into the left main coronary artery (4.98 ± 1.69 versus 3.97 ± 1.27 cm^2, $p < 0.01$). It is concluded that the site of injection of the contrast agent significantly influences the determination of area at risk. Therefore, data obtained by the two techniques should not be used interchangeably, and in a given study the area at risk should be measured consistently using one technique.

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Recent studies (1–5) have demonstrated that the left ventricular area at risk for necrosis after coronary occlusion can be accurately defined in a single tomographic plane or for the entire left ventricle using myocardial contrast echocardiography. Depending on the experimental design, investigators have employed different techniques for defining the risk area. Some (4,5) have injected the contrast agent proximal to the site of occlusion (into the left main coronary artery or aorta) and defined the risk area as that with no contrast effect (negative risk area). Others (3) have injected the contrast agent directly into the occluded vessel via a catheter whose distal end is positioned just beyond the site of the coronary occlusion and have defined the risk area as the area with contrast enhancement (positive risk area). The risk areas thus determined have been shown to correlate well with independent measures of risk area such as technetium autoradiography and intracoronary injection of colored dye (3–5). It has not been shown whether these two techniques provide identical information on the size of the area at risk. The purpose of this study, therefore, was to compare estimates of the area at risk for a single tomographic plane calculated by both methods.

Methods

Animal preparation. Six mongrel dogs weighing 23 ± 3 kg (mean ± 1 SD) were anesthetized with intravenous sodium pentobarbital (30 mg/kg body weight), intubated and ventilated with a Harvard respirator. A median sternotomy was performed and the heart suspended in a pericardial cradle. The left main coronary artery, the middle portion of the left circumflex artery and a distal branch of the left anterior descending artery were carefully dissected free from surrounding tissues and ties were placed loosely around them. A 20 cm long, 22 gauge polyethylene catheter (Deseret Corporation) was placed in the left circumflex artery with its tip positioned 1 to 2 mm beyond the location of the

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preplaced tie. The proximal end of this catheter was connected to a three-way stopcock. A similar catheter was placed in the distal branch of the left anterior descending artery and secured using the preplaced tie. The proximal end of this catheter was connected to a fluid-filled pressure transducer (Gould P 23I, Statham Corporation) and pressure in the left anterior descending artery was recorded using a multichannel recorder (Sanborn 7700, Hewlett Packard Corporation). An 8F catheter was introduced into the right femoral artery and advanced in a retrograde manner into the descending aorta. This catheter was then attached to a Silastic tubing with a 3/16 inch (0.48 cm) internal diameter (Dow Corning Corporation), which was connected to a Gregg cannula by means of a roller pump. This system was primed with 0.9% sodium chloride solution and the Gregg cannula was introduced into the ascending aorta by way of the left common carotid artery (Fig. 1). The roller pump was then started at a flow rate of 100 ml/min. The tip of the Gregg cannula was carefully introduced into the left main coronary artery and firmly secured there using the preplaced proximal tie. The roller pump was then adjusted so that the pressure measured in the left anterior descending artery after cannulation was similar to that at baseline.

Two-dimensional echocardiographic studies. These studies were performed using a commercially available mechanical sector-scanning system with a 5 MHz transducer (ATL, Mark III). Images were recorded on videotape using a ½ inch (1.27 cm) VHS recorder (Panasonic NV 8200). The transducer was fixed at the midpapillary muscle level using a clamp affixed to the procedure table. The gain settings were optimized at the beginning of the study and kept constant throughout the recording period. In all studies, a saline bath acted as an acoustic interface between the heart and the transducer. This was achieved by attaching the edges of a polyethylene sheet to the sternal edges and suspending it to cover the anterior surface of the heart. This plastic trough was filled with 0.9% sodium chloride solution (5).

Contrast agent. An agitated mixture of equal amounts of saline and 18.5 g/50 ml of diatrizoate meglumine/diatrizoate sodium (Renografin-76, E.R. Squibb) was used as a flow marker based on its ability to enhance echo intensity as described by Tei et al. (3).

Area at risk determination. The recorded images were analyzed on a commercially available off-line computer system (Microsonics, Easy View II, Microsonics Corporation). The video recordings were initially reviewed to select cycles in which post-contrast injection images were optimal. Selected cycles were transferred to a video disk system (Sony SVM 1010, Sony Corporation) and the end-diastolic frames of the cycles showing the best delineation of the area at risk using each method were selected for analysis. Figure 2 illustrates an example of a positive risk area and the corresponding negative risk area. Both positive and negative risk areas were measured and expressed in absolute terms as square centimeters and in relative terms as a percent of the entire myocardial area included in the particular short-axis image using a method similar to the one we previously reported (5). The endocardial and epicardial extents of the negative and positive risk areas were also measured (Fig. 2) and expressed both in centimeters and as percent of the endocardial and epicardial circumferences of the short-axis slice.

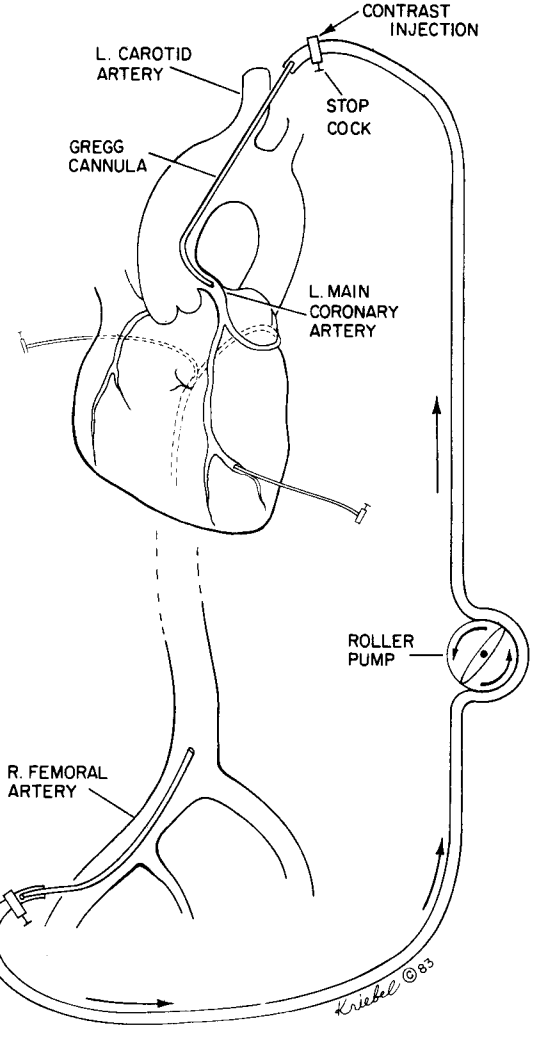

Figure 1. Diagrammatic representation of the animal preparation used in our study (see text for details). L = left; R = right.

Experimental protocol. The left circumflex artery was occluded. After the pressure in the distal left anterior descending artery returned to baseline (approximately 10 minutes after occlusion), 2 ml of contrast agent was injected into the left coronary artery through the Gregg cannula in order to define the negative risk area (that area with no contrast enhancement). Subsequently, 0.5 ml of this agent was injected into the catheter in the left circumflex coronary artery to define the positive risk area (that area with contrast enhancement). The amount of dye injected into the two sites was determined during pilot studies in which it was found that 2 ml of contrast agent resulted in optimal visualization

of the negative risk area, whereas 0.5 ml best defined the positive risk area. To allow complete clearance of the contrast agent from the myocardium, 5 minutes were allowed to elapse between the first and second injections of contrast agent.

Statistical analysis. The risk areas and their endocardial and epicardial extents measured by the two methods were compared using analysis of variance with repeat measures (BMDP2V, Department of Biomathematics, University of California, Los Angeles, revised 1983). The repeat measures analysis of variance is a generalization of the paired *t* test that allows comparison of conditions in the same experimental subjects measured under more than two experimental conditions and also permits nonrandom assignment of subjects to experimental conditions. The standard one-way analysis of variance is based on the assumption of random sampling in each group being compared. This assumption does not hold in experimental designs where the same experimental subjects are observed under different conditions (6). The difference in means was considered significant at a p value of less than 0.05.

To establish interobserver variability for the area at risk estimated by each method, all the measurements of the area at risk were performed by two independent observers. One of the observers then repeated the measurements to establish intraobserver variability. Interobserver and intraobserver errors were expressed as the square root of the variance using an analysis of variance model (BMDP8V). The average values of two observations made by a single observer were considered for purposes of analysis. All group data were expressed as mean ± 1 SD.

Results

Comparison of positive and negative risk areas. Table 1A shows the areas at risk determined with both methods in each animal. The areas at risk are expressed in both square centimeters and as a percent of the total myocardial area for each short-axis view. In all cases, the positive area at risk is slightly larger than the negative area at risk. The mean positive area at risk was significantly larger ($p < 0.01$) than the mean negative risk area, whether expressed in square centimeters (4.98 ± 0.69 versus 3.97 ± 1.27 cm^2) or as a percent of the total myocardial area (30 ± 0.06 versus 24 ± 0.09%).

Table 1B demonstrates the endocardial and epicardial extents of the risk areas measured with both methods. Although the myocardial extent of the positive risk area is significantly larger than that of the negative risk area (5.55 ± 1.65 versus 4.30 ± 0.95 cm, $p < 0.05$), the endocardial extent is not larger (3.48 ± 0.68 versus 3.09 ± 0.67 cm, $p = 0.20$).

Observer variability. Interobserver and intraobserver correlation and error for measures of positive and negative risk areas and their endocardial and epicardial extents are listed in Table 2. The error is expressed as the square root of the variance between the separate observations. There was good interobserver ($r = 0.87$ to 0.99) and intraobserver ($r = 0.98$ to 0.99) correlation for all measurements. Interobserver error was small (0.37 to 0.42 cm^2 for area; 0.25 to 0.63 cm for endocardial or epicardial extent), as was intraobserver error (0.25 to 0.30 cm^2 for area; 0.17 to 0.66 cm for endocardial or epicardial extent).

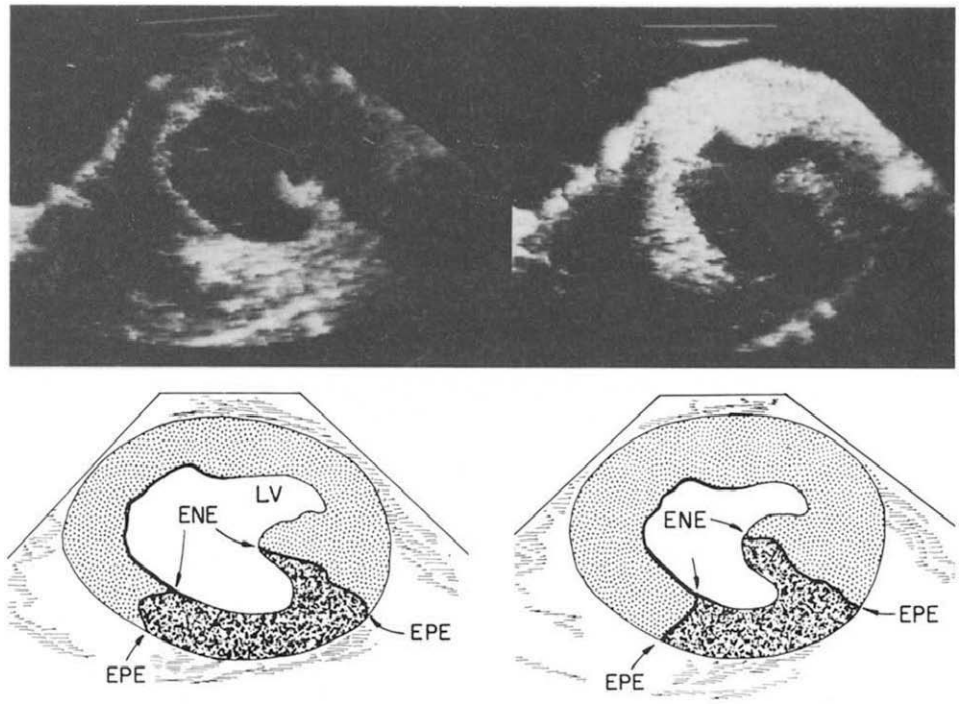

Figure 2. Top panel, Two-dimensional echocardiographic short-axis view at the papillary muscle level demonstrating area at risk after coronary occlusion when contrast agent was injected locally into the area at risk (positive risk area) **(left)**; and when it was injected into the left main coronary artery (negative risk area) **(right)**. **Bottom panel,** Diagrammatic representation of the positive and negative risk areas showing how the endocardial extent (ENE) and epicardial extent (EPE) of the risk areas were measured. LV = left ventricle.

Table 1. Comparison of Two Methods of Defining Risk Area

A. Area at Risk Measured by Both Methods

	Risk Area (cm^2)			Risk Area (% myocardium)		
Dog	Positive		Negative	Positive		Negative
1	8.15		5.70	39		36
2	4.40		3.11	34		24
3	3.41		2.95	20		14
4	4.95		4.37	27		24
5	5.12		5.07	34		31
6	3.83		2.61	28		15
Mean	4.98	← * →	3.97	30	← * →	24
± 1 SD	±1.69		±1.27	± 6		± 9

B. Epicardial and Endocardial Extent of Risk Area Measured by Both Methods (in centimeters)

	Endocardial Extent			Epicardial Extent		
Dog	Positive		Negative	Positive		Negative
1	3.3		3.68	8.39		5.70
2	3.05		2.53	5.05		4.11
3	2.45		2.54	3.29		3.24
4	3.68		2.99	5.61		3.82
5	4.52		4.14	5.80		5.20
6	3.80		2.67	5.13		3.72
Mean	3.48	← NS →	3.09	5.55	← † →	4.30
± 1 SD	±0.68		±0.67	±1.65		±0.95

*$p < 0.01$; †$p < 0.05$. NS = not significant.

Discussion

Two methods for defining area at risk for necrosis. Contrast myocardial echocardiography offers the investigator the unique opportunity to determine the area at risk for necrosis immediately after acute coronary occlusion in the intact beating heart. Previous reports have demonstrated that this measurement can be made for a single tomographic section of the left ventricle (3–5) or for the entire left ventricular myocardium (5). Depending on the experimental design, however, the method of defining risk area has differed. In one method, contrast medium is injected proximal to the site of occlusion and the risk area is defined as the area with no contrast enhancement (negative risk area). In the other method, contrast medium is injected into the occluded vessel and the risk area is defined as that demonstrating contrast enhancement (positive risk area). Although the risk areas defined by both methods have been shown to correlate well with independent measures of risk area such as technetium autoradiography or the intracoronary injection of colored dye (3–5), no study has compared the risk areas obtained using the two techniques of myocardial contrast enhancement. Our results demonstrate that 1) the area at risk is slightly but significantly larger when the contrast agent is injected locally into the occluded vessel than when it is injected proximally into the left main coronary artery; and 2) the greatest degree of overlap occurs at the epicardial margins of the risk areas. There are several possible physical and physiologic explanations for these observed differences.

Physical properties of ultrasound. One obvious physical explanation for the differences in positive and negative risk area relates to the lateral resolution artifacts inherent in all ultrasound systems. This lateral resolution artifact or point spread function permits only targets that are separated by more than the width of the ultrasound beam to be resolved as distinct and, for a scanning beam, spreads the echoes from individual point targets to the effective beam width for a given depth and power output. This point spread function should result in an apparent expansion of the positive risk area and a corresponding diminution of the negative risk area as noted in this study. Because these studies were conducted in open chest dogs, at a relatively low power output and with the area of interest relatively close to the focal zone of the transducer, the point spread function should be roughly equal for the endocardial and epicardial borders of the contrast zone. This phenomenon, therefore, could explain the mean differences in the two risk areas but cannot by itself explain the differences in the endocardial and epicardial extent of the contrast zones.

A second physical phenomenon that could influence the relative size of these risk areas is "blooming" of the con-

Table 2. Interobserver and Intraobserver Correlation and Error

Variable	Correlation	p Value	SEE	Error
		A. Interobserver		
Risk area				
Positive	0.98	<0.001	0.37 cm^2	0.37 cm^2
Negative	0.94	<0.005	0.40 cm^2	0.42 cm^2
Epicardial extent				
Positive	0.99	<0.0001	0.18 cm	0.25 cm
Negative	0.87	<0.05	0.59 cm	0.32 cm
Endocardial extent				
Positive	0.96	<0.005	0.24 cm	0.25 cm
Negative	0.93	<0.01	0.34 cm	0.63 cm
		B. Intraobserver		
Risk area				
Positive	0.99	<0.0001	0.22 cm^2	0.25 cm^2
Negative	0.99	<0.0001	0.19 cm^2	0.39 cm^2
Epicardial extent				
Positive	0.98	<0.001	0.16 cm	0.17 cm
Negative	0.94	<0.005	0.40 cm	0.66 cm
Endocardial extent				
Positive	0.99	<0.0001	0.05 cm	0.1 cm
Negative	0.99	<0.0001	0.08 cm	0.41 cm

SEE = standard error of the estimate.

trast signal on the display tube. As the video brightness is set to optimize the display of the noncontrast-enhanced echocardiographic images, the injection of contrast medium (which produces echoes significantly brighter than those from the image before injection of contrast medium) tends to increase the electron beam intensity in the regions of contrast, expanding the area of phosphor that is illuminated. This, like the point spread function of the ultrasound instrument, causes apparent encroachment on the negative contrast zone and expansion of the positive contrast zone. Again, this phenomenon should affect the endocardial and epicardial regions equally, given that the contrast density in those areas is the same. It should not produce the differences in the endocardial and epicardial extents of the risk area noted in our study.

Alteration in myocardial perfusion during ischemia. Several anatomic and physiologic differences in the positive and negative risk areas may account for the disparity between the endocardial and epicardial extents of the two contrast zones. In acute myocardial ischemia, there is a relatively greater reduction in endocardial blood flow compared with epicardial flow, in part because of more extensive epicardial collateral vessels. Therefore, when contrast medium is injected locally into the occluded vessel, it should travel farther outward radially on the epicardial surface by way of these collateral vessels. These same collateral vessels might be expected to transport contrast medium farther inward toward the center of the ischemic zone when the medium is injected into the left main coronary artery. This extension of contrast medium through the epicardial collateral vessels could explain the difference in the epicardial extent of the positive and negative risk areas. Our experimental model probably accentuates this effect in that, in our preparation, a fixed amount of blood is delivered to the Gregg cannula. Occlusion of the left circumflex artery thus causes increased flow to the left anterior descending coronary artery. This results in an immediate increase in the left anterior descending artery pressure that returns to baseline level within about 10 minutes, probably because of a change in the capacitance of the unoccluded coronary bed. It is therefore possible that, in this model, increased flow in the unoccluded bed causes the negative risk area to be somewhat smaller than it would be if total flow to the coronary bed was kept constant.

Pressure of contrast injection. The size of the positive risk area may be influenced by the pressure with which contrast medium is injected into the ischemic zone. Although we employed only a 0.5 ml bolus, if the pressure with which it was injected was high enough to drive the contrast medium through the epicardial collateral vessels beyond the actual ischemic zone, it would result in a relative expansion of the positive risk area. However, when the injection is made into the left main coronary artery, the contrast medium travels to the perfused area at a pressure similar to the normal coronary perfusion pressure. It is possible, therefore, that the injection of contrast into the left main coronary artery may more truly reflect coronary perfusion during acute ischemia.

Implications. Thus, our findings do not disagree with previously published data that validated the ability of both

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myocardial contrast echocardiographic techniques for determining the area at risk. As the differences between the positive and negative risk areas demonstrated in our study were small, the values obtained with either method would be expected to correlate equally well with independent measures of risk area. The fact that positive and negative echo contrast give slightly but significantly different measures for risk area, however, makes it reasonable to ask which is the more appropriate. Because there does not appear to be a reference standard with greater spatial resolution, this question cannot be answered at present. If the differences in the two techniques relate only to physical phenomena (lateral resolution artifacts and blooming), then for an equal contrast intensity in the two zones, the true border of the area at risk lies midway between the borders delineated by the positive and negative contrast techniques. If contrast crosses from one zone to the other through epicardial collateral vessels, however, the positive and negative contrast zones provide slightly different information and the difference may vary with the model.

Conclusions. Measurement of area at risk by myocardial contrast echocardiography is dependent on the site of injection of the contrast agent. This makes it important to use the same method consistently in an experimental setting, especially when the effects of interventions on risk area are being evaluated. Furthermore, data obtained by the different methods of contrast injected should not be used interchangeably.

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