LABORATORY INVESTIGATION
CORONARY ARTERY DISEASE

Hydrogen peroxide contrast echocardiography: quantification in vivo of myocardial risk area during coronary occlusion and of the necrotic area remaining after myocardial reperfusion

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ABSTRACT During sustained coronary occlusion in canine preparations, the extent of regions that fail to show contrast enhancement when imaged by supra-aortic hydrogen peroxide contrast echocardiography (SHPCE) has been shown to correlate well for single cross sections with the extent of malperfused myocardium "at risk" of infarction. In the present study, SHPCE was investigated as a means of determining the fraction of total left ventricular mass at risk during occlusion. Since necrotic tissue has low blood flow even when reperfused, we also investigated the potential of quantitating the extent of infarcted myocardium by measuring the extent of contrast defects seen with SHPCE performed during reperfusion. In 20 dogs the fraction of the left ventricle showing a contrast defect during coronary occlusion correlated well with the fraction of left ventricular mass "at risk" by an autoradiographic technique (autoradiography = 0.83 echocardiography + 8.6%; r = .89, SEE = 4.5%). SHPCE was also performed after 3 hr of reperfusion following occlusions varying in duration from 60 to 150 min. The fraction of the ventricle showing a contrast defect during reperfusion predicted the infarcted portion of the left ventricle as shown by triphenyl tetrazolium chloride staining (%left ventricle infarcted = 0.81 echocardiography + 3.3%; r = .84, SEE = 5.3%). Observer variability for the fraction of the ventricle showing a contrast defect was excellent during both occlusion and reperfusion. The ratio of the left ventricular extent of contrast-negative regions during reperfusion and occlusion was used to calculate a necrosis-to-risk index in vivo that correlated relatively well with the myocardial necrosis-to-risk ratio determined morphologically (r = .77, SEE = 16%). The results of this study indicate that SHPCE may be capable of determining not only the extent of myocardium at risk during acute coronary occlusion but also the extent of myocardial necrosis that occurs despite early reperfusion.


SUPRA-AORTIC hydrogen peroxide contrast two-dimensional echocardiography (SHPCE) is a useful method of determining the extent of myocardial perfusion defects in vivo during acute myocardial infarction in animals. Studies by Kemper et al.1 and Armstrong et al.2 performed during sustained coronary occlusion show close agreement between the cross-sectional extent of echocardiographic contrast defects and the extent of malperfusion and infarction determined morphologically.

Several animal studies have indicated that, because of local edema and microvascular damage, regional blood flow to necrotic myocardium remains severely depressed even when flow is restored through the previously occluded coronary artery.3-5 Flow to previously ischemic but viable tissue returns toward normal over the ensuing 3 days.5 Can SHPCE be used to quantitate infarct size after reperfusion by measuring the extent of associated regions of decreased echo contrast enhancement? Since early reperfusion appears to be the most effective means of obtaining significant tissue salvage after acute coronary occlusion, a method that documents in vivo the amount of tissue salvaged by reperfusion is potentially of great clinical importance.

To determine the applicability of contrast echocardiography to the problem of measuring infarct size after reperfusion, SHPCE was performed during and after progressively shorter periods of transient coronary occlusion in a canine preparation. Three contrast

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echocardiographic measures of myocardial perfusion were compared with their morphologic counterparts:

1. The fraction of left ventricular mass showing an echocardiographic contrast defect during occlusion (ECD,) was compared with that shown to be "at risk" by autoradiography.

2. The fraction of mass showing an echocardiographic contrast defect during reperfusion (ECD) was compared with that shown to be necrotic by histologic staining.

3. The ratio of ECD to ECD was compared with the morphologic ratio of necrotic mass to mass at risk as an index of myocardial salvage.

Methods

Experimental animal preparation. After preliminary evaluation to ensure adequate two-dimensional echocardiographic cardiac imaging, 20 mongrel dogs were anesthetized with 20 mg/kg iv pentobarbital, intubated, and placed on a Harvard respirator. The heart was exposed through a standard left thoracotomy. The left femoral artery and vein and the left atrium were cannulated. The left anterior descending artery proximal to the first diagonal (10 dogs) or the circumflex coronary artery distal to the first obtuse marginal (10 dogs) was dissected free. Five minutes after intravenous injection of 1.5 mg/kg lidocaine, the coronary artery was occluded with a Schwartz vascular clamp. Five minutes after occlusion an additional 1 mg/kg lidocaine bolus was given. The edges of the thoracotomy were approximated with surgical clips.

To produce infarcts of varying transmural extent, the duration of occlusion was maintained for four different intervals: 60 min (n = 5), 90 min (n = 5), 120 min (n = 5), and 150 min (n = 5). The chest was reopened at the end of the occlusion period.

A modification of the method of DeBoer et al. was used to assess the region of hypoperfusion during occlusion. Two million highly radioactive (0.5 mCi/kg) 99mTc-labeled albumin microparticles, 20 μm in diameter (3M Co.), were injected into the left atrium just at the completion of the occlusion period. Five minutes later, 1 mg/kg lidocaine was administered intravenously, the clamp was released, and the edges of the thoracotomy were reapprroximated. Animals were maintained 4 to 5 hr after reperfusion and were then killed by injection of potassium chloride. The heart was removed and the left ventricle was dissected free from surrounding tissue. After freezing with liquid Freon, the ventricle was sectioned parallel to the atrioventricular groove into 5 mm transverse sections. To define the extent of infarction, the slices were incubated in 1% triphenyl tetrazolium chloride (TTC) in phosphate buffer for 10 min then dipped in buffered formalin to enhance contrast between viable tissue, which stained dark red, and infarcted tissue, which appeared pale yellow. The pattern of staining on the basal side of each slice was traced on an acetate overlay and the slices were photographed.

The extent of the hypoperfused zone was determined on the same slices. The slices were placed basal side down on a sheet of high-speed x-ray film (Chronex 4; E. I. DuPont). Image contrast was improved by the use of sensitive x-ray contrast-enhancing screens. The film was exposed for 4 to 6 hr and developed (X-omat Automatic Processor). The epicardial and endocardial outlines of the tissue slices were transferred from the TTC tracings to a second set of acetate overlays. The zone of hypoperfusion was traced from the appropriate image on the developed film after the overlays were oriented to coincide with the autoradiograms by use of the anterior and posterior right ventriculoseptal junctions and papillary muscle contours in the perfused region as landmarks.

The area at risk in vivo (i.e., the area hypoperfused by autoradiography) was determined for each tissue slice by planimetry with a commercial light pen system (Irex Cardio-80). The sum of the percent of the area involved for each slice multiplied by its respective weight and divided by the total ventricular weight yielded the fraction of the left ventricle at risk by autoradiography (AR auto). The necrotic fraction of the left ventricle (Nec tissue) was similarly calculated from TTC tracings. The fraction of myocardium that was necrotic despite reperfusion was defined as NEC tissue/AR auto.

All values are expressed as percent.

Echocardiographic technique and analysis. Two-dimensional echocardiographic imaging and contrast injection techniques are described in detail elsewhere. Briefly, the left ventricle was imaged in the standard closed-chest position from the right sternal border. A Varian 3000 two-dimensional ultrasonograph with a 2.25 mHz phased-array transducer was interfaced with an on-line commercial color videodensitometer (Colorado Video), which allowed optimal homogeneous baseline echocardiographic setting for gain, depth, compression, and reject. Animals were imaged at four levels (mitral valve, high papillary, low papillary, and apex) during occlusion and 2%/ to 3%/ hr after reperfusion. During imaging at each level, a contrast injection of a fresh mixture of 2.0 to 3.0 ml 0.3% H2O2 and 1 ml autologous blood was flushed into the ascending aorta via a No. 8F pigtail catheter that had been placed above the aortic valve by fluoroscopic guidance. A total of eight to 10 contrast injections were given over the course of each experiment. Recordings were made with a Panasonic NV3160 reel-to-reel video recorder on Scotch 361 1/2 videocassette tape for subsequent analysis.

Echocardiographic analysis was performed by an observer blinded to the results of morphologic analyses. Recordings were played back and the series of contrast injections for occlusion or reperfusion was observed in real time. A representative frame occurring at end-diastole was traced for each level. Care was taken to choose a frame occurring in end-expiration at the echocardiographic level of the baseline settings for that injection. Traced frames were taken, if possible, within 15 sec after injection. Recordings were then played back in real time to verify accurate placement of epicardial and endocardial borders.

The fraction of the area showing echo contrast defect (%ECD) for each slice was determined by planimetry. The percent of left ventricular mass not enhanced was calculated with a formula that allocates a fraction of left ventricular mass to each echocardiographic level according to morphologic data derived from 10 previously studied animals: % left ventricle involved = 0.36 (%ECD mitral valve) + 0.27 (%ECD high papillary) + 0.23 (%ECD low papillary) + 0.14 (%ECD apex). (See appendix for raw data used to calculate this algorithm.)

Assessment in vitro: microbubble size. The mixture of dilute hydrogen peroxide and blood solution in a syringe and the intravascular injection of this echo contrast agent requires less than 15 sec. Preliminary observation of syringes of this mixture disclosed the appearance of grossly visible bubbles in solution by 30 sec after mixing. The progressive nature of the reaction...
between hydrogen peroxide and blood prevented accurate assessment of bubble size by direct sampling from the syringe. To simulate the conditions present at the time of injection, two drops of fresh heparinized blood were added to four drops of 0.3% hydrogen peroxide solution that had been previously placed on a prepared microscope slide. The solution was mixed with the tip of a needle and covered with a slip. The multiple fields were examined on a standard calibrated binocular microscope under 100 × magnification. Observations were made for 3 min with allocation of bubble size made at 30 sec intervals.

**Data analysis and reproducibility.** Data were tabulated and correlated and estimating errors were determined with a standard desk-top calculator (Litton Monroe 325 Scientist). Trends were analyzed by a standard one-way analysis of variance. All group data are expressed as mean ± SD.

To determine the reproducibility of the method, contrast studies during occlusion and during reperfusion were reanalyzed for 10 animals 4 months later by the first observer as well as by a second observer. During occlusion the correlation coefficient between observations for the first observer was .96 with an SEE of 3.2% and a mean difference between observations of 2.9 ± 2.8%, while the correlation coefficient between observers was .96 with an SEE of 3.2% and a mean difference between observers of 3.2 ± 2.5%. During reperfusion the correlation coefficient between observations for the first observer was .97 with an SEE of 2.0% and a mean difference between observations of 1.3 ± 1.6%, while the correlation coefficient between observers was .92 with an SEE of 3.4% and a mean difference between observers of 2.6 ± 2.6%.

**Results**

**Tissue analysis.** Mean left ventricular weight was 113.2 g (range 78.2 to 154.6). Table 1 presents group data for tissue analyses of region at risk, region infarcted, and percent salvage for the four occlusion times. For all animals the region at risk as measured by autodiography averaged 27.3 ± 9.8% of the left ventricle and ranged from 9.7% to 43.7%. There was no significant difference in risk area between the four groups. The portion of the ventricle infarcted averaged 13.6 ± 9.7% (range 2.6% to 35.8%). There was a significant increase in the left ventricular fraction infarcted as the duration of occlusion increased from 60 to 150 min (p < .01). The region infarcted despite reperfusion was 48.5 ± 26.6% of the area at risk (range 11.1% to 88.0%). The fraction infarcted was directly related to the duration of occlusion before reperfusion (p < .001).

Hemodynamic data for the four groups are presented in table 2. For all animals there was a small but significant decrease in heart rate during the reperfusion period (p < .05). There were no significant intragroup differences in heart rate, blood pressure, or left atrial pressure during occlusion or reperfusion.

**Hemodynamic effects and microbubble size.** A total of 143 supra-aortic injections of hydrogen peroxide were made during occlusion and reperfusion. As in our previous experience there were no alterations in heart rate or left atrial pressure during injection. There was no visible alteration in left ventricular contraction pattern.

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**TABLE 1**

**Morphologic data**

<table>
<thead>
<tr>
<th>Occlusion time (min)</th>
<th>LV wt (g)</th>
<th>Tissue at risk (%LV)</th>
<th>Tissue necrosis (%LV)</th>
<th>Tissue necrosis Tissue at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>119 ± 21</td>
<td>26 ± 11</td>
<td>5 ± 11^A</td>
<td>19 ± 6^B</td>
</tr>
<tr>
<td>90</td>
<td>116 ± 30</td>
<td>31 ± 9</td>
<td>11 ± 6^A</td>
<td>37 ± 20^B</td>
</tr>
<tr>
<td>120</td>
<td>105 ± 15</td>
<td>24 ± 12</td>
<td>16 ± 10^A</td>
<td>62 ± 20^B</td>
</tr>
<tr>
<td>150</td>
<td>119 ± 17</td>
<td>28 ± 9</td>
<td>23 ± 9^A</td>
<td>76 ± 9^B</td>
</tr>
</tbody>
</table>

LV = left ventricular.

^a p < .01 for trend from 60 to 150 min occlusion.

^b p < .001 for trend from 60 to 150 min occlusion.

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**TABLE 2**

**Group hemodynamic data during occlusion and reperfusion for each occlusion time**

<table>
<thead>
<tr>
<th>Occlusion time (min)</th>
<th>HR (mmHg)</th>
<th>BP (mmHg)</th>
<th>LA (mmHg)</th>
<th>Reperfusion HR (mmHg)</th>
<th>BP (mmHg)</th>
<th>LA (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>145 ± 7</td>
<td>108 ± 8</td>
<td>7 ± 5</td>
<td>132 ± 17</td>
<td>106 ± 8</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>90</td>
<td>148 ± 26</td>
<td>95 ± 19</td>
<td>4 ± 2</td>
<td>144 ± 24</td>
<td>84 ± 7</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>120</td>
<td>146 ± 26</td>
<td>107 ± 16</td>
<td>4 ± 2</td>
<td>132 ± 14</td>
<td>97 ± 23</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>150</td>
<td>149 ± 21</td>
<td>116 ± 24</td>
<td>8 ± 4</td>
<td>129 ± 39</td>
<td>108 ± 30</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Mean</td>
<td>147 ± 21</td>
<td>106 ± 18</td>
<td>6 ± 4</td>
<td>134 ± 24^A</td>
<td>99 ± 19</td>
<td>6 ± 4</td>
</tr>
</tbody>
</table>

HR = heart rate; BP = mean arterial pressure; LA = left atrial pressure.

^a p < .05 vs occlusion.
or ventricular size. Mean arterial pressure fell more than 5 mm Hg during nine injections. The maximal fall seen, 15 mm Hg, returned to baseline by 60 sec after injection. The frequency of ectopic activity, observed for 3 min before and after each injection, was not altered by contrast injection (2.4 ± 4.4 vs 2.2 ± 4.5 beats/min during occlusion and 10.3 ± 15.9 vs 10.6 ± 14.7 beats/min during reperfusion — two animals with accelerated ventricular rhythm throughout reperfusion were excluded from calculations).

In the studies in vitro of microbubble size, a total of 20 observations were made with the blood from four different animals. A total of 299 bubbles were sampled in the initial 30 sec period. Mean microbubble size during this interval was 50 ± 24 μm (range 10 to 120). Mean observed bubble size increased during the ensuing time intervals to 62, 75, 88, 83, and 85 μm at 60, 90, 120, 150, and 180 sec, respectively.

\[ \text{mean size} = 50 \pm 24 \text{ μm} \]

Relationship of echocardiographic contrast defects in vivo to tissue measures of risk, infarction, and salvage. Two-dimensional echocardiograms of normal myocardial sections during supra-aortic injection of hydrogen peroxide showed transmural contrast enhancement (figure 1). During occlusion, regions that failed to

**FIGURE 1.** Normal hydrogen peroxide contrast echocardiographic study at the high papillary muscle level. Baseline study (left) shows clear endocardial and epicardial target definition with few intramyocardial echoes. After peroxide injection (right), normal myocardium is enhanced. Orientation is shown on the left (S = septum; A = anterior; L = lateral; P = posterior).

**FIGURE 2.** Representative contrast echocardiographic, autoradiographic, and tissue sections at the high papillary muscle level from an animal undergoing 120 min of circumflex occlusion. Contrast echocardiography during occlusion (top left) disclosed a sharply bordered region of decreased contrast extending from the posterior to the lateral wall (between arrows). During reperfusion (top right) the contrast-negative region (between arrows) is smaller and more irregularly defined. The percent of the area of the section that failed to show enhancement during occlusion, 32.6%, coincides with 38.3% defined by autoradiography (bottom left). The contrast-negative area during reperfusion, 28.2%, coincides with the 26.7% of this section defined as infarcted by TTC staining (bottom right). (Orientation as in figure 1.)
show contrast enhancement were well defined and were generally transmural (figure 2). The portion of the ventricle that did not show contrast enhancement during occlusion (echo occluded) averaged 22.5 ± 10.6% (range 8.6% to 43.2%). Although contrast defect during occlusion underestimated the autoradiographic fraction of the ventricle at risk by 4.2 ± 5.6%, the correlation between echocardiographic and tissue estimation of region at risk in vivo was good (% left ventricle at risk: autoradiography = 0.83 echocardiography occluded + 8.6%; r = .89, SEE = 4.5%). When animals were grouped according to site of occlusion, similar correlation was seen for both the left anterior descending (autoradiography = 0.99 echocardiography occluded + 5.99%; r = .93; SEE = 3.4%; figure 3, left) and circumflex groups (autoradiography = 0.76 echocardiography occluded + 9.4%; r = .88, SEE = 5.1%; figure 3, right).

During reperfusion contrast studies, regions with decreased contrast enhancement were irregular in shape and frequently nontransmural in a major portion of their areas (figure 2). Regions of contrast defect during reperfusion were located within the area at risk and were consistently circumferentially smaller than those seen during occlusion (figure 2). During the reperfusion period, the portion of the ventricle that failed to show contrast enhancement in vivo averaged 12.6 ± 10.0% (range 0% to 32%). The fraction of the left ventricle that did not show contrast enhancement during reperfusion (ECD,) correlated reasonably well with the fraction of the left ventricle that subsequently was shown to be necrotic by TTC staining (% left ventricle necrosed = 0.81 echocardiography reperfused + 3.3%; r = .84, SEE = 5.3%; figure 4).

An echocardiographic index of myocardial salvage in vivo comparable to the morphologic ratio of necrotic and risk areas was derived by comparison of the previously determined contrast echocardiographic measures of necrosis and risk. The ratio of the fraction of the ventricle that failed to show contrast enhancement during reperfusion, representing necrosis, and the fraction that failed to show enhancement during occlusion, representing risk, was 0.58 ± 0.43. A significant correlation was seen between echocardiographic
and morphologic necrosis-risk ratios (tissue necrosis/risk = 0.47 ECD/ECD₀ + 21%; r = .77, SEE = 16.9%; figure 5).

Discussion

This study confirms previous work from our laboratory¹ and that of others,² indicating that the size of regional echocardiographic contrast defects, observed in vivo during the supra-aortic injection of a dilute hydrogen peroxide and blood solution, closely parallels the extent of regions of abnormal myocardial perfusion. In our previous work, contrast studies were performed 6 hr after coronary occlusion. Echocardiographic contrast defect size, examined at four echocardiographic levels, was highly predictive of the cross-sectional extent of malperfusion and infarction demonstrated morphologically.¹ This study extends our prior work by examining the value of contrast echocardiography as a means to predict the fraction of the left ventricle that is malperfused at a time early in the course of infarction when therapeutic intervention is likely to result in significant salvage. The fraction of the left ventricle that was not contrast enhanced was highly predictive of the fraction of left ventricular myocardial mass that was malperfused as measured by autoradiographic examination of radioactive microspheres delivered by left atrial injection during occlusion. The extent of this "risk area" was predicted well by SHPCE during occlusion of both the left anterior descending and circumflex coronary arteries.

The second phase of this study was designed to determine whether the phenomenon of limited reperfusion known to occur in infarcted myocardium could be detected and quantitated as a region of decreased echocardiographic contrast enhancement in studies performed during reperfusion. In preliminary experiments we observed that contrast injections performed within 1 hr after the release of occlusion resulted in profound increase in contrast in the epicardial layers of ischemic tissue, which overshadowed any decreases in contrast that might have been present in the endocardial layers. Increased contrast enhancement in the epicardial layers, which we believe represents reactive hyperemia in previously ischemic but not infarcted tissue, was no longer manifest 3 hr after release of the occlusion. Contrast defects at that time were irregular in shape and were located within the region that had been defined as at risk during occlusion. Contrast defects during reperfusion were usually nontransmural over a large portion of their areas. The fraction of the left ventricle that failed to show contrast enhancement 3 hr after reperfusion correlated well with the fraction of left ventricular mass that was infarcted as indicated by TTC staining.

The pattern of contrast enhancement we observed is compatible with the results of regional blood flow studies of White et al.⁵ In infarctions reperfused after 2 to 24 hr of occlusion, they observed microsphere-determined blood flow during occlusion and after 1 and 72 hr of reperfusion. In the core area, which showed 95% to 100% necrosis histologically, blood flow was approximately 10% of normal at all measured times. In the ischemic zone, which showed 82% to 90% necrosis, flow was decreased by only 20% after 1 hr of reperfusion but was decreased to 30% to 40% of control and approached occlusion levels by 72 hr of reperfusion. In the marginal and jeopardized zones, which showed approximately 50% and 5% necrosis, respectively, flow was not different from that in control tissues at any point in the reperfusion period. These data indicate that a shift in blood flow away from predominantly infarcted tissue toward viable tissue occurs during the first 72 hr after occlusion. Although we found a relatively good correlation between the contrast-negative area during reperfusion and infarct size in studies performed 3 to 4 hr after the release of occlusion, data from White et al.⁵ suggest that more optimal separation between infarcted and viable tissues might be obtained in contrast studies performed after 24 or 72 hr of reperfusion.

FIGURE 5. Relationship of morphologic necrosis-risk ratio ("Tissue Infarcted/Tissue Malperfused") to echocardiographic necrosis risk ratio in vivo determined as the ratio of contrast-negative left ventricular fractions during reperfusion and occlusion (Echo Contrast defect reperfused/occluded). Regression line is solid and line of identity dashed. Auto = tissue malperfused by autoradiography. See legends to figures 3 and 4 for other abbreviations.
Although the fraction of the left ventricle that failed to show contrast enhancement after 3 hr of reperfusion was, in general, predictive of the fraction of the ventricular mass infarcted, as indicated by subsequent TTC staining, caution must be used in interpreting the data for individual cases. In two animals the echocardiographic contrast defect substantially overestimated the transmural extent of infarct size. Although the data cited above from White et al. suggest that the local changes that result in depressed regional flow during reperfusion are manifested in predominantly infarcted tissue, no work has been done that explores in greater detail the precise topographic nature of reperfusion to infarcted and previously ischemic but uninfarcted tissue. Our data suggest that local factors in previously ischemic but uninfarcted tissue may limit blood flow during reperfusion. This may lead to an overestimation of infarct size in echo contrast studies performed during the early reperfusion period in some animals; however, our studies indicate that animals that show normal contrast enhancement of previously ischemic regions in studies performed during the early reperfusion period have morphologic evidence indicating that only a very small fraction of the ventricle has become infarcted. The long-term functional importance of the finding of normal contrast enhancement during reperfusion of previously ischemic areas in terms of the recovery of contraction in that area is a subject of current research.

The third phase of this study utilizes the contrast-negative ventricular fractions during reperfusion and occlusion to derive an index of necrosis to risk areas in vivo and compares it to accepted morphologic measures. A significant correlation coefficient of .77 was seen between contrast echocardiographic and tissue morphologic measures of myocardial salvage; however, the standard error of the estimate at 16.9% is too large to allow highly precise classification with this technique.

One potential area for improvement has been mentioned: altering imaging time during reperfusion. However, another source of random variability in the relationship between in vivo and morphologic measures may be unavoidable. While the injection of radioactive microspheres and the contrast studies for assessment of risk area were performed concomitantly, the autoradiographic examination could not be performed until after the 4 hr reperfusion period. Several studies indicate that myocardium reperfused after a period of prolonged ischemia becomes edematous, particularly when infarcted. Haendchen et al. noted a 56% increase in regional myocardial thickness when ischemic tissue was reperfused after 3 hr of occlusion. Edema present in the previously ischemic area would tend to artifactualy expand the risk area as assessed by the radioactive microsphere technique and may explain to a large extent the 4.2% underestimation of the fraction of the ventricle at risk autoradiographically as assessed by size of echocardiographic contrast defect. The variability in expansion of the autoradiographic risk area is magnified when the risk area is used as a denominator in the calculation of the morphologic necrosis-risk ratio. Edematous expansion of ischemic and infarcted tissue may also explain to some extent the overestimation of morphologic necrosis-risk ratios as determined by contrast echocardiography, since the risk areas are determined by echocardiography before expansion. In three animals the fraction of the ventricle infarcted as indicated by contrast echocardiography during reperfusion actually exceeded the fraction at risk indicated during occlusion (figure 5).

**Strengths and limitations of the method.** SHPCE is subject to the limitations inherent in all echocardiographic methods. Although rapid acquisition of high-quality real-time images is easily accomplished in most subjects, some may be suitable for only limited echocardiographic examination. Most previous echocardiographic studies relating to infarct size have analyzed only single cross-sectional slices. In our study, aggregate analysis of four cross-sectional levels was performed to derive the fraction of left ventricular mass involved. This approach, which samples regional perfusion throughout the left ventricle, is less dependent on exact reproduction of previous views and is likely to reflect more accurately the heterogeneous nature of coronary artery disease than those that rely on a single two-dimensional section. The four cross-sectional views used are easily obtained in dogs; however, in a substantial number of patients, it may not be possible to obtain all of the cross sections necessary to apply the present ventricular reconstruction algorithm.

The major limitation of previous echocardiographic approaches to the quantification of acute myocardial ischemia has been reliance on the analysis of myocardial performance as an indicator of tissue viability. During acute coronary occlusion, functional analysis has been shown to overestimate and correlate relatively poorly with the extent of infarction. Wall motion analysis is least accurate during the first few hours after occlusion when interventions aimed at myocardial salvage are most likely to be beneficial. After reperfusion, viable myocardium may remain ‘‘stunned’’ for a period of time. Several investigators have found no correlation between myocardial func-
tion and salvage in the first 48 hr after reperfusion of previously ischemic myocardium.\textsuperscript{12,17} One of the important advantages of SHPCE is that it is not dependent on myocardial function for analysis.

One potential limitation of the present method is the resolution of the echocardiographic system, a first-generation Varian 3000 system with a 2.5 mHz phased-array transducer. Two-dimensional echocardiographic resolution is in the range of 1 to 2 mm in the plane perpendicular to the transducer face; however, because of beam spread, resolution at the lateral edges of the sector scanned may be as poor as 3 to 5 mm. At contrast borders the high-intensity echoes reflected from peroxide-generated oxygen microbubbles may encroach on adjacent nonperfused tissue. Although the relationships between contrast enhancement and tissue measures of risk and infarction are linear with a slope close to unity, contrast enhancement tends to underestimate morphologic measures, particularly for small regions. Small risk areas, which tend to be transmural, are more easily detected than small subendocardial infarctions, which tend to be irregular and may have a transmural dimension at the limits of the echocardiographic system. The problem of defining contrast borders may be particularly difficult in the lateral regions of the sector where dropout and nonhomogeneity in the intensity of reflected ultrasound is particularly marked.\textsuperscript{18} The reproducibility for the definition of the extent of regions that failed to show contrast enhancement in our study was very good; however, methods that require the quantitation of the amount of contrast enhancement rather than its presence or absence may be particularly difficult, especially in the lateral zones. Resolution may be improved by the use of newer systems that utilize 3.5 or 5 mHz focused transducers. Poststudy processing, particularly digital subtraction techniques,\textsuperscript{19} may also improve the definition of regions of enhancement if problems with accurate registration of images can be overcome.

The mixture of a dilute solution of hydrogen peroxide and blood results in the production of oxygen microbubbles. The exact size and number of these microbubbles is a complex function of the concentration of hydrogen peroxide present initially, the duration of time since mixing, the concentration of peroxide present in solution, and the rate of resorption of the formed oxygen microbubbles by the blood stream. Our data indicate that the microbubble size at the time of injection is approximately 50 \( \mu \)m (range 10 to 120). Although these microbubbles are relatively large when compared with the minimal diameter of capillary vessels (8 \( \mu \)m), their size is only one two-hundredth of the maximum resolving power of available echocardiographic instruments. Data from radioactive microsphere studies indicate that particles of this size may not be distributed uniformly across the myocardial thickness. Domenech et al.\textsuperscript{20} noted a gradient in radioactivity of 2.5:1 for the distribution of uniform 50 \( \mu \)m microspheres between the endocardial 1 to 2 mm and the epicardial 1 to 2 mm of the myocardial thickness. They suggest this gradient is the result of axial streaming of larger microspheres, with trapping in the endocardial end arterioles. Although we have noted that contrast enhancement persists in the endocardium well beyond that seen in the epicardium,\textsuperscript{1} we have not found a gradient in the intensity of contrast enhancement between epicardium and endocardium using the far more heterogeneously sized microbubble produced by hydrogen peroxide. The endocardial persistence seen, which may represent vascular resorption of trapped oxygen microbubbles, may last for as long as 5 min but does not appear to result in any hemodynamic or contractile consequences. The heterogeneity of bubble sizes and persistence of endocardial contrast may limit the applicability of hydrogen peroxide microbubbles to the analysis of myocardial defects in perfusion and may make it inappropriate for use in techniques that measure the "washout" of contrast as a means of quantitating regional perfusion.\textsuperscript{21}

SHPCE is applicable at present to the study of coronary occlusion and reperfusion in experimental animals. Transfer of this technique into the clinical arena awaits further tests of safety. Gaffney et al.\textsuperscript{22} in this country and Wang et al.\textsuperscript{23} in China have safely used doses of dilute hydrogen peroxide similar to ours as an echocardiographic contrast agent during right-sided injections in over 100 patients. We have performed more than 200 hemodynamically monitored supra-aortic injections in animals. Most show no alteration in heart rate and left atrial or aortic pressure. In our prior experiments,\textsuperscript{1} the maximum fall in blood pressure in any animal (18 mm Hg) returned to baseline by 60 sec after injection. Ambient left ventricular ectopic activity was unaltered by dilute peroxide injection even during reperfusion, which is known to be a possible arrhythmia-inducing setting. Our experience is similar to that of Gaffney et al.,\textsuperscript{22} who noted no alterations in hemodynamics or alertness in five conscious dogs during left atrial injection of up to 10 ml of 0.2\% solution. Further careful clinical evaluations should be undertaken to determine whether supra-aortic injection of hydrogen peroxide or another contrast agent can be safely used as a means of obtaining myocardial echocardiographic contrast enhancement in human beings.
SHPCE allows repeated delineation in vivo of the extent of malperfused myocardial regions. During coronary occlusion the fraction of the ventricle that fails to show contrast enhancement is highly predictive of the fraction of left ventricular mass that is sufficiently hypoperfused to be “at risk” of infarction. Three hours after reperfusion the extent of regions that fail to show enhancement echocardiographically correlates well with the fraction of ventricular mass infarcted morphologically. The ratio of regions failing to show enhancement during reperfusion to those during occlusion provides an approach to the quantification of myocardial salvage in vivo. Experience with dilute hydrogen peroxide as an echocardiographic contrast-enhancing agent in an animal preparation demonstrates the potential of contrast echocardiography for the evaluation in vivo of myocardial infarction in human beings.

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References


APPENDIX

Morphologic data used to calculate algorithm for allocation of fraction of left ventricular mass to each echocardiographic level

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Mean % 14.2 ± 4.4

AP = apex; LP = low papillary muscle; HP = high papillary muscle; MV = mitral valve.

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Hydrogen peroxide contrast echocardiography: quantification in vivo of myocardial risk area during coronary occlusion and of the necrotic area remaining after myocardial reperfusion.

A J Kemper, J E O'Boyle, C A Cohen, A Taylor and A F Parisi

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