EXPERIMENTAL STUDIES

Echocardiographic Detection of Ischemic and Infarcted Myocardium

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The purpose of this study was to determine the potential of a clinically adaptable two-dimensional echocardiographic system using computer enhancement and a mathematically defined integrated backscatter ratio for the early detection of ischemic and infarcted myocardium. Fifteen dogs had two-dimensional echocardiograms recorded during either open chest coronary occlusion (n = 5), closed chest occlusion (n = 5), occlusion followed by reperfusion (n = 3) or sham coronary occlusion (n = 2). A serial increase in integrated backscatter ratio, representing differences in returned ultrasound intensities between a reference point and specific myocardial regions, was detected between 7 and 12 minutes of complete occlusion in 9 of 12 animals (p < 0.05), and at minutes 18, 43 and 67 in the remaining 3 animals. Reperfusion after 20 minutes of occlusion in two studies resulted in normalization of the backscatter ratio. An increase in backscatter ratio was not detected when 5 minute occlusion periods were used or during the 5 hour sham occlusion studies.

The computer enhancement techniques utilized in this study provided increased visual detail of intracardiac structures over that provided by routine two-dimensional echocardiograms; myocardial tissue was identifiable in what appeared to be echo-free segments; and boundaries that appeared as noncontiguous horizontal lines on the routine echocardiograms were identifiable as trabeculae.

The results indicate that: 1) significant increases in backscatter from nonperfused myocardium are detectable echocardiographically within 12 minutes of coronary occlusion and temporal changes can be assessed in the canine model, and 2) the echocardiographic data acquisition and computer analysis system utilized provide a clinically adaptable approach to identify and map myocardial characteristics in human beings.

Myocardial scar can be detected with clinically available ultrasound instrumentation (1-4). It has been proposed that earlier and more subtle tissue changes of ischemia and evolving infarction also should be detectable ultrasonically if more detailed assessments of reflected ultrasound signals were available (5).

Sound waves returning from tissue examined by pulsed ultrasound contain distinct echoes reflected without major distortion (specular reflection) from structures that are relatively large compared with the dominant wavelength in the pulse, as well as lower intensity echoes generated from very small structures that scatter the input energy in a multidirectional fashion (6). The term backscatter is used to denote the reflected ultrasonic energy after scattering within the tissue has occurred. The characteristics of these relatively low level signals generally are considered to provide information about the finer structure of the ultrasound target.

In vivo quantitative measurements of ultrasonic backscatter from hypoperfused myocardium have been reported using reflected ultrasound and integrated backscatter (7,8); integrated backscatter denotes spectral data averaged over frequency. Mimbs et al. (7) demonstrated quantitative changes in integrated backscatter as early as 1 hour after coronary artery ligation in open chest canine studies (7). Cohen et al. (8) performed both closed and open chest studies in the same animals. They found that if integrated backscatter data recorded from the closed chest studies were corrected for a chest wall attenuation-frequency function, a significant change between control values and those recorded at 2 to 4 hours after coronary artery ligation could be demonstrated (8). In their studies, areas of interest in the myocardium were located and positioning of the backscatter window was accomplished utilizing M-mode echocardiographic techniques.

The purpose of this study was to determine if a mathematically defined integrated backscatter ratio, derived non-
invasively through the incorporation of an internal calibration measurement and coupled with computer enhancement of clinical two-dimensional echocardiograms, would provide a sensitive and specific approach for the detection and temporal assessment of underperfused myocardium. The goal was to expand the clinical application of ultrasound in characterization of myocardial tissue.

Methods

Animal Studies

Fifteen adult mongrel dogs of either sex and weighing 20 to 25 kg were anesthetized with pentobarbital (20 mg/kg body weight), intubated and ventilated with a Harvard respirator using room air and supplemental oxygen. Before instrumentation, immediately before and after coronary occlusion and periodically throughout each study, two-dimensional echocardiographic studies (as described later) were performed and recorded on 0.5 inch (1.27 cm) video tape. Continuous recording of the electrocardiogram, aortic blood pressure and Doppler left circumflex coronary artery flow was done throughout each study using an eight channel (Gould Brush 2800) recorder. Arterial blood gases were monitored and maintained within normal physiologic range.

Open chest studies. Eight of the 15 dogs had open chest studies via a right thoracotomy incision in the fifth intercostal space for exposure of the heart. Either a hydraulic occluder (four dogs) or umbilical tape (four dogs) was placed around the left circumflex artery distal to the first marginal branch. A Doppler flow crystal also was secured just distal to the occluder site. Complete two-dimensional echocardiographic studies were performed in the closed chest state before surgery and with the transducer coupled directly to the epicardial surface in the open chest state. The transducer was hand-held in six studies and supported by a rigid mechanical arm in two studies. Coronary artery occlusion was maintained in five of the eight open chest studies for 4 (n = 2), 5 (n = 1) or 6 (n = 2) hours before sacrifice of the animals.

Closed chest studies. The remaining seven dogs had closed chest studies. The chest was entered through an incision in the right fifth intercostal space and the pericardium was incised. The left circumflex coronary artery was then isolated and a hydraulic occluder was placed distal to the first marginal and exteriorized between the scapulae. A Doppler flow crystal was also placed just distal to the occlusion site. Bicillin, 1 million units, was given intramuscularly immediately before and at 48 hours after surgery. These animals were allowed to recover for 7 to 10 days before coronary occlusion studies were performed. During occlusion studies, the ultrasound transducer was hand-held in six of the seven studies. The coronary artery occlusion was maintained in five of the seven closed chest dogs for 4 (n = 2), 5 (n = 1) or 6 (n = 2) hours before sacrifice of the animals.

Control group. Two of the 15 dogs served as control animals, one for the open chest studies and the other for the closed chest studies. A hydraulic occluder and Doppler flow crystal were placed around the left circumflex artery of each dog and all procedures described for open and closed chest occlusion studies were performed except for coronary artery occlusion. Echocardiographic studies were performed over 5 hours in each study with the transducer hand-held.

Reperfusion studies. Two dogs in the open chest group and one in the closed chest group had reperfusion studies. In one open chest study the left circumflex coronary artery was completely occluded over a 7 second period and the occlusion was maintained for 5 minutes, released for 30 minutes and reinstituted for 5 minutes. The dog was then sacrificed. Continuous echocardiograms were recorded using a hand-held transducer throughout the control stage and the 40 minute study period. For the other two studies, occlusion using a hydraulic occluder (closed chest) or umbilical tape compression (open chest) was completed within 7 to 10 seconds and maintained for 20 minutes. After 30 minutes of reperfusion, the occlusions were repeated and maintained for 20 minutes; the dogs were then sacrificed. Continuous echocardiograms were recorded using a hand-held transducer throughout the control stage and 70 minute studies.

Echocardiographic studies. On the basis of probable blood flow distribution of the vessel selected for occlusion, the posterior papillary muscle region was designated as most likely to become ischemic; therefore, short-axis echocardiographic recordings at the papillary muscle level were made continuously beginning immediately before occlusion and through the first 30 minutes; 3 to 5 minute recordings from this region were then made every 15 minutes for the next hour and then every 30 minutes up to 6 hours after occlusion. No changes were made in gain/ramp settings once control levels were set and a Polaroid photograph was taken of the time gain compensation ramp for each study. An electrocardiographic limb lead II was recorded continuously on videotape and displayed on the echocardiographic slave monitor. Before sacrifice, guide wires via spinal needles were placed as guided by the echographic monitor image in order to transect the 30° echographic view (Fig. 1).

Histologic preparation. The hearts were excised rapidly, rinsed free of blood and cut into approximately 1 cm thick slices perpendicular to the interventricular groove. Incubation in nitroblue tetrazolium, a yellow diformazan salt that stains blue in the presence of dehydrogenase (9) or the dehydrogenase coenzyme nicotinamide adenine dinucleotide, or both (10), was used to stain and demarcate noninfarcted tissue in the 10 dogs having 4 to 6 hour oc-
Inclusions and in the 2 control dogs. For these 12 studies, myocardium that failed to stain histochemically was considered "infarcted." Triphenyltetrazolium-chloride was used as a marker for perfused tissue in the three reperfusion studies. A 10% solution of triphenyltetrazolium-chloride was injected into the left atrium just before sacrifice. Areas receiving blood appeared bright red and areas not receiving blood appeared pale red in comparison.

Transparent graph paper was placed over the region of the excised hearts bordered by the guide wires. Myocardial borders within the guide wires and areas of stained ("normal") myocardium were manually outlined for later visual comparison with computer reconstruction and mapping of the two-dimensional echograms.

**Echographic Data Acquisition System**

A clinically adaptable off-line computer system that provides both quantitative and qualitative analysis of two-dimensional 30° echocardiograms (11) was utilized for these studies. This system accesses and directly digitizes the returned ("raw") ultrasonic signals as opposed to utilizing the fully processed signal that standard clinical systems display on the video monitor. An 8 bit analog to digital converter digitizes, in stop-mode, the raw two-dimensional analog signals at sampling rates of 2 to 10 MHz. For this study, the 2 MHz rate was used and each stop-frame of displayed two-dimensional echographic data was a half-frame containing 54 lines; 480 points were sampled for each line. The absolute intensity at each point was determined and stored in a disk data file. A DEC PDP11/45 interactive

**Figure 1.** Guide wires, positioned before the animal was sacrificed, transecting the echographic view are visible postmortem.

**Figure 2.** Left ventricular short-axis echograms recorded serially from a closed chest animal using a hand-held transducer. Numbers refer to minutes after coronary artery occlusion.
Figure 3. Left ventricular short-axis echogram as seen on the clinical echocardiographic video monitor. IVS = left side of the interventricular septum; LV = left ventricle; PM = papillary muscle; PW = posterior left ventricular wall.

computer program provided visual and hard copy plotting of all 54 lines of data both before and after the data transformations described below had been made.

A Smith-Kline Ekoline I mechanical sector scanner was used to record and store the standard two-dimensional 30° echocardiograms on 0.5 inch (1.27 cm) videotape. A Printronix printer-plotter was used for hard copy plots and a Lexidata Video-Graphics system was used for color video displays of the digitized signal.

Data transformations. The following data display formats were made available by this system: 1) histograms of the returned signal intensities for each of the 480 points on each of the 54 lines in the stop-frames; 2) plots of those portions of the raw signal frames needed to reproduce the standard two-dimensional video monitor displays; 3) contour maps of the intensities of the returned signals; 4) plots of ultrasound intensities for regions of interest within the 30° sector scan; and 5) integrated backscatter (E) ratio plots within specific regions of interest of the myocardium.

The integrated backscatter ratio \( E_R \) is the quotient of the integrated backscatter at a given location \((r, \theta)\) divided by the integrated backscatter at reference point \(r_o \) (\(E_o\)):

\[
E_R = \frac{E(r, \theta)}{E_o}.
\]

Backscatter over a 0.5 mm radius centered about the posterior left ventricular endocardium was selected as the reference point \((r_o)\) because it includes the specular reflections from the posterior left ventricular endocardium. This is advantageous because: 1) the influence of angulation, gain settings, beam path and attenuation is minimal; 2) earlier pilot studies had shown the clear delineation of the posterior left ventricular endocardium, numerically, as compared with cavitary blood; 3) this area is in close proximity to the region expected to be influenced by left circumflex artery occlusion; and 4) measurements of integrated backscatter have been shown to be reliable even in the presence of altered left ventricular wall motion and systolic wall thinning (that is, negative thickening) \(8\). The posterior endocardial echo also was used for boundary identification and guidance in the placement of the backscatter window.

The integrated backscatter ratio for this study was calculated from echo intensities, \(I(r, \theta)\), and backscatter energy \(E\) over 0.5 mm \(\delta\) areas located at: 1) posterior left ventricular endocardial boundary \((r_o)\); 2) posterior left ventricular epicardial/pericardial boundaries; and 3) between endocardial and epicardial boundaries at radius \(r\) and angle \(\theta\).

The backscatter ratio at any instant \((t)\) in the experiment was defined as:

\[
E_R(t) = \frac{E(r(t), \theta(t))}{E_o}.
\]

where

\[
E_o = \int_{r_o}^{r_o + \delta} I^2(r, \theta)dr
\]

and

\[
E(r, \theta) = \int_{r}^{r + \delta} I^2(r, \theta)dr.
\]

Thus, an integrated backscatter ratio of 1.0 from endocardium to epicardium indicated that the echoes received throughout the myocardial tissue were of the same intensity as the specular endocardial intensity. An integrated backscatter ratio of zero indicated that no low intensity echoes over a distance of approximately 0.5 mm \(\delta = \pm 0.25\)
Isolated areas for computer analysis, that is, ischemic, infarcted and normal myocardium, were operator-selected via placement of (visible) cursors to define the segments of interest in the computer plots (Fig. 4). The operator was guided by the transparent overlay of the myocardial tracing and the known pathologic composition of the tissue in selecting specific segments for analysis.

_Histogram plots were used_ to assess and compare distributions of intensities between varying tissue types as subsequently identified from the pathologic studies. Computer-reconstructed echograms were color-displayed initially in point mode, using all of the reflected echoes and then using only those echoes required to reproduce the stop-frame as it appeared on the video monitor. Contour maps of the stop-frames, based on absolute intensity levels, were used to display tissue boundaries. Plotting of the operator-selected areas of interest provided an enlarged image of the areas for visual study and expedited multiple contour analyses of smaller segments within the area. This aided in establishing the validity of the computer-identified transition zones. A cursor was also used by the operator to verify that the integrated backscatter window was located within computer-identified tissue boundaries.

_Line selection and validation_ were based on agreement between each of the 54 sector lines selected numerically by the computer and displayed on the Lexidata monitor, with each of 54 lines drawn by the operator at equal distances.
throughout the 30° transparent overlay of myocardium bounded by the transecting guide wires.

Values for integrated backscatter ratios were analyzed and averaged for a "region" comprised of three adjacent lines (from $\theta_1$ to $\theta_3$) and from differing distances (from $\lambda_0$ to $\lambda_1$) for 1) full thickness areas (endocardium to epicardium), and 2) partial thickness areas. This allowed areas of known tissue types to be isolated and compared serially throughout the study.

The average backscatter ratio ($\overline{E}$) from endocardium to epicardium ($\lambda$) for three given lines ($\theta$ at a specific time $t$) was defined by:

$$\overline{E}(t) = \frac{1}{\theta_1 - \theta_3} \int_{\theta_1}^{\theta_3} \int_0^1 E_R(\lambda, \theta) \, d\lambda d\theta.$$
Results

There were no statistically significant differences in variables analyzed from studies derived from a hand-held transducer versus rigid support of the transducer. Hand-held transducer studies were technically easier to perform. Echocardiograms recorded serially using a hand-held transducer are shown in Figure 2.

Myocardial infarction studies. Myocardial infarction, as determined by nitroblue tetrazolium staining, involved the posterior papillary muscle and adjacent posterior left ventricular wall in all five open chest studies. The region of posterior wall infarction extended from base to apex in three of the open chest studies. Closed chest study dogs had infarction involving 1) the posterior papillary muscle and adjacent posterior left ventricular wall in three dogs, 2) only the posterior left ventricular wall in one dog, and 3) a 1 cm × 1 cm × ¾ cm isolated inferomedial segment in one dog. More than 75% of all posterior myocardium within the 30° sector was infarcted in three cases compared with patchy areas of infarction in the other seven cases.

Reflective echocardiographic signals. Open chest studies. Posterior endocardial echoes averaged 31% (range 27 to 33) greater in intensity during baseline studies than did left ventricular cavity echoes (probability [p] = < 0.05). The integrated backscatter ratio control values were less than
0.85 at all levels within the posterior left ventricular myocardium. The epicardial/pericardial region within a 0.5 mm distance showed a ratio greater than 1.0 in all five dogs, indicating that the epicardial/pericardial interface was a stronger reflector than the endocardium. The average integrated backscatter ratio recorded over time from the infarcted tissue area (E) is shown in Figure 5A. A statistically significant (p < 0.05) directional increase in integrated backscatter was detectable within 10 minutes after occlusion in three of the five studies. Similar changes were not detected until 18 minutes (Dog 2) and 43 minutes (Dog 4) after occlusion in the remaining two animals.

The average integrated backscatter ratio recorded over the full thickness between endocardium and epicardium and averaged for three adjacent lines (E) is also shown in Figure 5 for lines traversing the infarcted zone (panel B), the histochemically heterogeneous zone (panel C) and normal tissue (panel D). All posterior myocardium within the 30° sector was infarcted in two cases (Dogs 2 and 4).

A directional increase in estimated integrated backscatter was detectable within 10 minutes of complete occlusion in four of the five studies (p < 0.05). Directional changes of similar magnitude were not detected until 67 minutes after occlusion in the remaining case (Dog 5). Averaging of sequential lines was not essential for the detection of significant serial changes in integrated backscatter ratio. Serial changes in backscatter ratio between control and 10 minutes after occlusion for three isolated lines selected to represent ischemic (line 10), mixed (line 23) and normal tissue (line 48) (Fig. 7) are shown in Figure 8.

Control studies. Each control animal was sacrificed after 5 hour sham studies. No areas of infarction were visible by nitroblue tetrazolium staining. No significant serial changes in integrated backscatter ratio were recorded; the averaged ratio (E) for lines 9, 10 and 11 (a), 22, 23 and 24 (b) and 47, 48 and 49 (c) throughout the 5 hours are shown in Figure 9 for each animal. No significant changes were seen when integrated backscatter ratio (E) was calculated for space-selected (λx = 25 and λy = 75) areas for these same lines.

Reperfusion studies. 5 minute occlusion. Complete occlusion was maintained for 5 minutes, released for 30 minutes and reapplied for 5 minutes in one open chest animal. No significant serial changes in integrated backscatter ratio were detected even though triphenyltetrazolium-chloride staining validated lack of blood flow to the posterior left ventricular wall (Fig. 10). Averaged backscatter ratios (E) for lines 22, 23 and 24 at specified times throughout the study are shown in Figure 11.

20 minute occlusion. Complete occlusion was maintained for 20 minutes, released for 30 minutes and reapplied for 20 minutes in two dogs; one open chest and one closed chest. A significant increase (p < 0.05) in integrated backscatter ratio was detected at 7 minutes in the open chest dog and at 12 minutes in the closed chest animal. Both animals showed that the backscatter ratio normalized at 30 minutes after release of occlusion and increased significantly (p < 0.05) with reocclusion.
Values for averaged integrated backscatter ratio \( \bar{E} \) recorded over the full thickness between endocardium and epicardium for three adjacent lines traversing the nonperfused area in each animal during occlusions are shown in Figure 12.

**Discussion**

The results of this study indicate that: 1) significant increases in reflected ultrasonic backscatter recorded from nonperfused myocardium are detectable echocardiographically as early as 7 to 12 minutes after complete coronary occlusion, and 2) it is feasible to use a clinically adaptable echocardiographic data acquisition and computer analysis system to study myocardial ischemia in the intact dog without the necessity for external calibration with a "perfect reflector."

**Origin of increased backscatter early after coronary occlusion.** Although the results do not define the mechanisms responsible for the observed increase in integrated backscatter early after coronary occlusion, as the backscatter ratio is derived from reflected echo intensities, an increase in backscatter from within the myocardium might be explained by: 1) the presence of more laminar surfaces, or 2) greater acoustic differences between interfaces, or both. Relative to the former, arteriolar and capillary collapse secondary to the absence of blood flow would result in more laminar surfaces and thus more "specular" reflectors. Edema formation within the first 10 minutes of occlusion also might be involved through the creation of greater acoustic differences between interfaces. Differences in the temporal onset of detectable backscatter increases are perhaps attributable to differences in collateral blood flow, and the prompt reversibility with reperfusion favors the arteriolar capillary collapse hypothesis in our opinion.

**Advantages of the present methods.** Our findings support the data of Cohen et al. (8), who showed an increase in integrated backscatter at 2 to 4 hours after coronary ligation. They did not report data recorded earlier after coro-
Figure 9. Averaged integrated backscatter ratio (\(E\)) recorded from echo lines 9, 10 and 11 (a), 22, 23 and 24 (b), and 47, 48 and 49 (c) during two sham occlusion studies.

Figure 10. Pathologic specimen showing result of injecting triphenyltetrazolium into the left atrium just before sacrifice. Perfused areas appear dark. Averaged integrated backscatter data from this animal are shown in Figure 11.

Figure 11. Averaged integrated backscatter ratio (\(E\)) recorded from posterior left ventricular wall during baseline control (C), at 5 minutes after complete occlusion, at 30 minutes after reperfusion (R) following release of occlusion and at 5 minutes after reocclusion. No significant changes in \(E\) were detected.

Figure 12. Averaged integrated backscatter ratio (\(E\)) recorded before coronary occlusion (stage I), after complete occlusion (stage II), after reperfusion (R) (stage III) and after reocclusion (stage IV). Numbers refer to minutes in each stage.

Our primary ligation. Our method differs from theirs in that: 1) invasive techniques are not used to correct for the influence of canine chest wall, 2) two-dimensional echocardiography is used for area localization rather than M-mode echograms, and 3) integrated backscatter is calculated using reflected intensities from a mechanical two-dimensional echo transducer as opposed to using a separate backscatter transducer.

The use of two-dimensional echograms combined with internal calibration from the heart itself reduces the inherent limitations attending the use of sound waves for tissue interrogation in human beings. Because the internal backscatter reference point radially neighbors the region of interest and because the control value (\(E_0\)) for the backscatter reference point is reestablished for each temporal study, influences of chest wall thickness, cardiac motion and contractile patterns are minimized. Factors that alter \(E_0\) also may affect \(E_1\), yet the ratio would remain constant. The need
to correct the backscatter measurement for chest wall thickness is thus bypassed and feasibility for use in studies of ischemia in human beings is enhanced. Our experimental data suggest that a serial increase in integrated backscatter ratio might indicate an active process, a serial decrease (an improved or lessening ischemic effect) and a relatively unchanging abnormal backscatter ratio (a stable or inactive process). It is proposed that the ability to objectively evaluate specific myocardial segments of interest will ultimately enable tracking of an ischemic process in a localized area.

**Clinical implications.** No consistently accurate means are available to differentiate reversible from irreversible ischemic injury or for tracking the ischemic process clinically. The method we describe is a promising start toward realizing those goals. We attribute the relatively early detection of increases in backscatter ratio to the computer enhancement and analysis system used. Storage of the digitized data allows greater flexibility in terms of window settings and analysis of frequency content in multiple formats and comparisons at varying time intervals. Our computer analysis technique uses video-recorded signals, therefore the echo data acquisition system is adaptable to recording at the bedside.

Once the precise mechanisms for the changes in backscatter are proven and the digital processes defined, the digital processes required for backscatter analysis can be incorporated into echographs using 80 to 90° mechanical sector scanning transducers. Furthermore, computer enhancement and display of all returned echoes coupled with integrated backscatter ratios from a region of interest can be accomplished using a microprocessor, thereby broadening the possibility for meaningful echocardiographic tissue assessments at the bedside.

**References**