Contrast ultrasound in the assessment of patients presenting with suspected cardiac ischemia

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Echocardiography is a portable technology that can be used to assess myocardial, pericardial, and valvular structure and function. Doppler echocardiography provides the ability to evaluate blood flow in large vessels and within cardiac chambers. Recently, the advent of microbubble contrast agents, which can opacify the systemic circulation, has improved the ability of echocardiography to evaluate left-ventricular function by improving delineation of the left-ventricular endocardial border. Furthermore, these microbubbles can be used to assess myocardial perfusion and quantify myocardial blood flow. Myocardial contrast echocardiography has been studied in multiple clinical situations, including the acute evaluation of patients presenting with suspected cardiac ischemia. Ongoing research is focused on the development of microbubbles that are capable of detecting molecular and cellular events within the circulation, which may allow distinction of acute vs. remote ischemic insults to the myocardium. This multifaceted technology promises to be of increasing clinical utility—not only for the heart, but for any organ accessible to ultrasound. (Crit Care Med 2007; 35[Suppl.]:S280–S289)

Ultrasound imaging has become ubiquitous across the world, because of its portability, noninvasive nature, high spatial and temporal resolution, low cost, and lack of ionizing radiation. In cardiac applications, echocardiography can be used to assess myocardial, pericardial, and valvular structure and function. Doppler echocardiography provides the ability to evaluate blood flow in large vessels and within cardiac chambers.

One of the most significant recent advances in echocardiography has been the development of microbubble contrast agents. These agents enhance the backscatter signal from blood, which has enabled the assessment of organ perfusion and microvascular flow with ultrasound. Further technological advances may allow the production of microbubbles that are capable of detecting molecular and cellular events within the circulation.

The framework for this review will be the setting of patients presenting with suspected cardiac ischemia, but this technology can be applied to any organ that is accessible to ultrasound.

Background

Microbubble Contrast Agents. Microbubbles are compressible and oscillate within a sound field, making them effective scatterers of ultrasound. Thus, although blood is usually 30 dB to 40 dB less echogenic than tissue, the presence of microbubbles can dramatically enhance the acoustic signals derived from the intravascular space. Commercially available microbubbles that are currently used clinically share some common properties; they are encapsulated by a thin shell, and are smaller than red blood cells (~3 μm in diameter). This allows microbubbles, which remain exclusively intravascular, to transit the microvasculature unimpeded. They contain a gas with high molecular weight and low solubility and diffusibility, so the microbubbles can opacify the systemic circulation after intravenous administration.

Unlike the tracers used with computed tomography, magnetic resonance imaging, or single-photon–emission computed tomography, microbubbles are hemodynamically inert, and have a microvascular rheology identical to that of red blood cells (RBCs) (2). These unique properties make microbubbles excellent blood-flow tracers. There are currently three agents in the United States approved for use in patients: Optison (GE-Amersham, Princeton, NJ), Definity (Bristol-Myers Squibb Medical Imaging, North Billerica, MA), and Imagent (IMCOR Pharmaceutical, San Diego, CA). All three are approved for left-ventricular (LV) cavity opacification and enhancement of LV endocardial border delineation.

Imaging Modalities for Myocardial Contrast Echocardiography. Over the last decade, novel imaging modalities have been designed specifically for myocardial contrast echocardiography (MCE). These were driven mainly by the need to produce unique microbubble signals that could be distinguished from tissue for perfusion imaging. These techniques take advantage of microbubbles oscillating in the sound field. At the correct frequency and acoustic power, microbubbles can be induced to oscillate nonlinearly, which produces microbubble signals that are distinct from myocardial tissue (3). At higher acoustic powers that are still well within the clinical range, microbubbles can be destroyed by ultrasound, a phenomenon that also generates nonlinear microbubble signals (4–6). The first MCE modality developed was harmonic imaging, where ultrasound at a particular frequency is transmitted, and a high-pass filter is used to selectively receive signals at double the transmit frequency (second harmonic). Harmonic imaging is an excellent modality for LV...
endocardial border delineation because it is available on all current systems, and provides a high frame rate. To avoid excessive microbubble destruction in the cavity, especially at the apex, it is important to reduce the acoustic power for LV endocardial border delineation applications. The associated reduction in signal can be compensated by increasing the receive gain. The gains should be high enough for the epicardial border to be seen, which allows the assessment of wall thickening as opposed to endocardial excursion, which can be affected by tethering from adjacent segments. More recently, low-power MCE techniques designed primarily for perfusion imaging have been used for LV endocardial border delineation, as well.

It is beyond the scope of this review to discuss all the perfusion-imaging modalities, but in brief, the main objective of these modalities is to optimize the microbubble signal-to-tissue noise ratio. Algorithms that incorporate receive filters, as well as the transmission of sequential ultrasound pulses of differing phase and/or power, have been used to generate unique “nonlinear” microbubble signals while suppressing tissue signals. Algorithms can be divided into those that use high acoustic power to destroy microbubbles to produce nonlinear signals, and those that merely induce nonlinear oscillations of microbubbles to do so (low-power techniques). A schema is provided in Figure 1 illustrating the various imaging modalities currently available on commercial systems. High-power techniques require imaging at a low frame rate (triggered to the heart rate) because they destroy microbubbles, while low-power techniques can be performed even at high frame rates, allowing LV function and perfusion to be assessed simultaneously.

A representation of backscatter signals obtained from microbubbles and tissue during high-power imaging is shown in Figure 2. Received backscatter signals from microbubbles (solid line, a) are of equal intensity to those from tissue (dotted line, b) at the fundamental frequency \(f_0\). At the second harmonic (2\(f_0\)), improvement in the signal-to-noise ratio (c vs. d) is achieved, but strong tissue clutter (d) is still present. Further enhancement in the signal-to-noise ratio (e vs. f) has been accomplished with ultraharmonic imaging (transmit at \(f_0\), with receive only at \(5/2f_0\)), which takes advantage of the inability of tissue to generate ultraharmonic frequencies (f) compared with microbubbles (e). Harmonic and ultraharmonic imaging use B-mode processing techniques to suppress tissue noise, but Doppler processing can also be used. With the latter, received signals from tissue show little spectral decorrelation compared with the transmitted signal, which can then be suppressed by an appropriate wall filter. Conversely, microbubble destruction produces frequencies that are markedly different from those transmitted, allowing microbubble backscatter signals to be effectively separated from tissue (power harmonic Doppler).

With low-power imaging techniques, tissue clutter is suppressed using multipulse schemes where the amplitude (power modulation) or phase (pulse inversion) of sequential transmit pulses is altered. With power modulation (Fig. 3), pulses at half-height (a) and full-height amplitude (b) are transmitted sequentially. The received signals from a linear scatterer such as tissue (Fig. 3a) are identical to the transmitted pulses. Subsequently, when the received echoes from the half-height pulses are scaled and subtracted from the full-height signal, effective removal of tissue clutter is achieved. On the other hand, the response of microbubbles to pulses of different power is nonlinear (Fig. 3b), which produces received signals that cannot be scaled and subtracted. With pulse inversion (Fig. 4), sequential pulses of inverted phase are transmitted. Again, linear scatterers (Fig. 4a) produce received signals identical to the transmitted pulses, and summation of the received signals results in cancellation of tissue noise. Nonlinear scatterers (Fig. 4b), however, generate signals that cannot be summed. Both power modulation and pulse inversion also have been combined into a single modality (contrast pulse sequencing), where alternate ultrasound pulses have differing amplitude and phase.

Quantification of Flow. At steady state, the inflow and outflow of microbubbles in any microcirculatory unit are constant, and are dependent only on the flow rate of microbubbles (or RBCs). Microbubbles within the myocardium can be destroyed with high-energy ultra-
sound, and rapidly eliminated from the myocardium (4). When imaging is subsequently performed to measure the rate of microbubble reappearance, RBC velocity can be determined (7).

To understand this concept mathematically, assume that all microbubbles within an ultrasound field are destroyed by a single ultrasound pulse and that the elevation (thickness) of the ultrasound beam \((E)\) is uniform (Fig. 5). If new microbubbles enter this field with a flat profile at a velocity \(v\), then the distance \((d)\) they will travel within the beam elevation as the pulsing interval is prolonged \((t_1 - t_4; \text{Fig. 5, } B-E)\) will be given by the equation \(E/vt\). Thus, as the time interval between consecutive destructive ultrasound pulses is increased, more time is allowed for microbubble replenishment, which is dependent on the velocity of the microbubbles. As pulsing interval lengthens, the rise in microbubble concentration is displayed as a corresponding rise in myocardial acoustic intensity (AI), provided that the relationship between AI and microbubble concentration remains linear. As shown in Figure 5\(F\), when the pulsing interval exceeds \(\tau\), AI will remain constant. This plateau phase will reflect the effective microbubble concentration within the myocardial microcirculation. At any given concentration of microbubbles, the AI at the plateau phase will be proportional to the sum of the cross-sectional area of microvessels within the beam thickness.

The model predicts a sharp demarcation between the upslope and plateau phases of the pulsing interval–AI relation (Fig. 5\(F\)). In reality, however, neither the beam elevation nor the degree of microbubble destruction is expected to be entirely uniform. The profile of the microbubbles also is not expected to be entirely flat. The actual relation between AI and pulsing interval is therefore more likely to be curvilinear and can be described by the exponential function \(y = A(1 - e^{-\beta t})\), where \(y\) is AI at a pulsing interval of \(t\), \(A\) is the plateau AI, and \(\beta\) is the rate constant that determines the rate of rise of AI.

The value \(A\) represents myocardial or capillary blood volume (MBV), and \(\beta\) represents myocardial blood flow velocity as follows: Because the slope of the tangent to the curve is given by \(dv/dt = A\beta e^{-\beta t}\), the slope at the origin \((t = 0)\) is \(A\beta\). As shown in Figure 5\(F\), this slope is also equal to \(A/\tau\). Therefore, \(\tau = 1/\beta\). Eliminating \(\tau\) between equations results in \(v = E\beta\). Thus, for a given beam elevation (thickness at a given distance from the transducer), the mean velocity of microbubbles is proportional to \(\beta\).

Because flow is a volume of blood moving at a certain mean velocity, the product of MBV \((A)\) and myocardial blood flow \((\beta)\) reflects myocardial microvascular flow: If flow, \(f\), occurs through an area, \(a\), then \(f = av\). If \(E\) is constant, then the value \(a\) will be proportional to \(A\). Therefore, with rearrangement substitution for \(v\) we get \(f = AE\beta\). If \(E\) is known, then \(v\) can be expressed in cm·s\(^{-1}\). Similarly, if the microvascular cross-sectional area is known, then \(A\) can be expressed in cm\(^2\). The product of \(A\) and \(v\) will then represent \(f\) in mL·s\(^{-1}\) (7).

In an experimental study, an excellent linear relationship was found between MBF measured with radiolabeled microspheres and MCE-derived MBF (7).
A pulsing interval of fitted to an exponential function. At the capillary level velocity of blood in the coronary vessels is approximately 8 million capillaries, of which contain 90% of the MBV (11). The human heart has approximately 8 million capillaries (10). The human heart has a coronary blood circulation, which predominantly consists of microcirculation (9). At baseline, approximately 8% of the MBV (A), and MBF (Aβ) in both experimental and clinical settings.

Evaluating the gradual replenishment of microbubbles into the myocardial microcirculation has become the de facto method for assessment of myocardial perfusion (MP) using MCE. The adult human coronary circulation has a coronary blood volume of approximately 45 mL. This is divided nearly equally among the arterial, venous, and microcirculatory networks (9). At baseline, approximately 8% of the LV mass is blood present in the microcirculation, which predominantly consists of capillaries (10). The human heart has approximately 8 million capillaries, which contain 90% of the MBV (11). The velocity of blood in the coronary vessels is related to their size. At the capillary level (mean length and diameter of 0.5 mm × 7 μm), the mean red-cell velocity at rest is about 1 mm·s⁻¹ (11). The thickness of the ultrasound beam (elevation dimension) is approximately 5 mm; thus, after microbubbles have been destroyed with a pulse of ultrasound, it takes about 5 secs for microbubbles to completely replenish the myocardial microcirculation within the beam elevation again. Consequently, the presence of normal resting myocardial blood flow can be clinically estimated by determining if confluent myocardial contrast enhancement is present after 5 secs of replenishment.

**Evaluation of Chest Pain Patients in the Emergency Department**

**Case Presentation.** EC is an 81-yr-old man with no prior cardiac history. His only cardiac risk factor was hypertension, which was well controlled with Atenolol. He was enjoying a pleasant afternoon at a casino with some friends. He started to notice substernal chest pressure as he was leaving. He says that he was not exerting himself very much, and he started noticing the pain shortly after eating. He had some associated shortness of breath, and also noticed some cramping in his left arm. Because the pressure persisted for an hour, he presented to the emergency department (ED). The physical examination was unremarkable. The initial electrocardiogram (ECG) did not show any significant ST-segment or T-wave changes. The patient was administered aspirin and nitroglycerin, with some improvement in the pain. The initial cardiac troponin I was negative. His chest radiograph was clear, with a normal mediastinum.

The typical management of such a patient would subsequently entail further treatment with nitroglycerin and morphine to relieve the chest pain, followed by serial serum cardiac marker determinations to diagnose or refute acute myocardial infarction (AMI). Meanwhile, ongoing ischemia is not being definitively treated and patients with noncardiac chest pain are forced to stay for a “rule out.”

This case illustrates the challenge of evaluating patients presenting to the ED with suspected cardiac chest pain (CP) and a nondiagnostic ECG. The physical examination and early laboratory data are neither sensitive nor specific for AMI (12, 13). Even though the majority of patients have minor causes of CP, most are admitted to hospital or an observation unit for an AMI to be “ruled out” (14, 15). Apart from the large economic burden imposed by this approach (current costs have been estimated at $10 billion in the United States alone), the inconvenience and loss of time for the patient is significant. Even with the use of “accelerated diagnostic protocols,” at least 2 negative serial serum cardiac markers are usually obtained during a minimum 6 hr to 12 h period of observation before “early” stress testing (16, 17). Thus, a test with excellent negative predictive value that can identify very-low–risk patients suitable for immediate stress testing or direct discharge from the ED would be extremely valuable. Conversely, the same test should identify high risk patients in whom admission and therapy could be started without delay. The assessment of regional function (RF) and MP using MCE may be such a test (18, 19).
Pathophysiologic Basis for MCE in the Assessment of Chest Pain Patients

Based on the ischemic cascade (20), reductions in MBF below normal resting levels will first be manifested by the development of a MP abnormality, and are followed within seconds by RF abnormalities (21). Figure 6 illustrates the close coupling that exists between resting MBF and wall thickening. As MBF decreases below the normal resting level of 1 mL·min⁻¹·g⁻¹, there is a commensurate decrease in wall thickening (22). It is not surprising, therefore, that the assessment of either perfusion or function (generally single-photon–emission tomography (23–25) for the former and echocardiography (24–26) for the latter) has been shown to be of considerable value in detecting AMI, and for risk-stratifying CP patients early after ED admission.

Utility of MCE in Chest Pain Patients

With the advent of MCE, both RF and MP can be assessed with echocardiography. Unlike single-photon–emission tomography, MCE provides an immediate assessment of MP without requiring time for tracer uptake or postprocessing; it is portable, has superior spatial and temporal resolution, and does not expose the patient to radiation. MCE enhances the assessment of RF by ensuring that all myocardial segments are well delineated so that subtle segmental abnormalities are not missed (27). The evaluation of RF with echocardiography is one of the most subjective and difficult skills to master, but the use of contrast for LV endocardial border delineation has been shown to improve reader confidence and observer agreement (28, 29).

We have recently completed a study in >1,000 patients evaluating the role of MCE in patients presenting to the ED with suspected cardiac CP and no ST-segment elevation on the initial ECG (18). Patients were followed for the development of cardiac-related death, AMI, unstable angina pectoris, congestive heart failure, and revascularization within 48 hrs of ED presentation. Of the 1017 patients studied, 166 (16.3%) had early events, the majority being AMI. As shown in Figure 7, the assessment of RF with MCE significantly increased the diagnostic information for predicting these events obtained from demographics (D), clinical risk factors (C), and ECGs (E) (Bonferroni method, p < .0001). When MP was added, significant additional diagnostic information was obtained (Bonferroni method, p = .0002).

Apart from the identification of ischemia, accurate risk stratification of CP patients is also important. Those who are intermediate or high risk for an adverse outcome may require admission to a critical care or telemetry unit, treatment with potent antiplatelet agents (30–32), or early referral for cardiac catheterization (33). We have shown that MCE can be used to provide earlier and more accurate triage of these patients than clinical evaluation with the thrombolysis in myocardial infarction (TIMI) risk score (34), which is derived from multiple clinical variables including cardiac troponin I. Because cardiac troponin I may not be elevated or immediately available at the time of patient presentation, complete risk stratification and initiation of therapy may be unnecessarily delayed.

We evaluated the prognostic utility of a score derived only from variables that are available immediately at the time of a patient’s presentation to the ED. Because laboratory results may not be received for many hours after the initial presentation, a modified TIMI risk score (mTIMI) that excluded cardiac troponin I was derived (maximum score of 6). Based on their mTIMI scores, patients were categorized as low (score ≤2), intermediate (score of 3 or 4), or high (score, ≥5) risk. Although patients with a low mTIMI score had the lowest incidence of primary events (24 (4.1%) still had an early AMI within 24 hrs of enrollment. Patients with an intermediate score had a similar event rate as those with a high risk score (11% vs. 8.9%; p = .71). Thus, the mTIMI score was unable to discriminate between these groups (19).

MCE, on the other hand, can be performed immediately at the bedside to evaluate RF and MP. The incidence of a primary (nonfatal AMI or total mortality) event within 24 hrs of enrollment was only 0.4% in patients with normal RF and MP. This is in comparison to a 2.3% AMI rate for patients with CP discharged from ED based on routine evaluation (35). The negative predictive value of MCE is therefore excellent. The ability of MCE to provide incremental early and late prognostic information is shown in Figure 8A for all patients with a low (0–2) mTIMI score and Figure 8B for all patients with an intermediate (3–4) mTIMI score. Follow-up was performed up to 2 yrs (mean, 8.7 months) from the time of enrollment. In both groups, RF and MP could subdivide patients into low-, intermediate-, and high-risk subsets (19).

Based on the data supporting the diagnostic and prognostic utility of MCE, we have worked closely with the ED to implement 24/7 coverage with on-call sonographers and level-III–trained echocardographers to provide MCE studies in any patient with suspected cardiac CP. We have also devised a protocol that incorporates echocardiography early in the management of patients with suspected cardiac CP.

Fortunately for EC, therefore, a MCE was requested shortly after his presentation to the ED. The RF study demonstrated a significant wall-thickening abnormality involving the mid and distal septum, anterior wall, and apex (Fig. 9A). MP within dysfunctional segments showed delayed replenishment of microbubbles (decreased resting RBF), which denotes the presence of reduced resting MBF from a critical epicardial coronary stenosis (Fig. 9). As shown in Figure 9B, the akinetic region demarcated by arrowheads in Fig. 9A shows little contrast enhancement at a pulsing interval of one cardiac cycle. At a pulsing interval of five cardiac cycles (Fig. 9C), the midepicardial and supraspical regions demonstrate...
some contrast enhancement, while the subendocardium remains hypoperfused. Only at the longest pulsing interval of 8 cardiac cycles (Fig. 9D) does the subendocardium start to show enhancement. The presence of confluent contrast enhancement of dysfunctional segments at long pulsing intervals, however, denotes the presence of significant residual myocardial viability (Fig. 9D). The patient was therefore rapidly triaged to the cardiology department and therapy was started for acute coronary syndrome. Cardiac troponin I subsequently became positive 8 hrs after the patient’s arrival in the ED, and cardiac catheterization revealed multi-vessel disease, which was successfully bypassed.

**Limitations of MCE**

There is a bias in the field of echocardiography that abnormal RF will only be detected in patients with ongoing CP. The etiology of cardiac CP may be due to AMI, unstable angina, or transient ischemia. In AMI, because most wall thickening is derived from the subendocardium, necrosis of even 20% to 30% of the transmural extent of the myocardium will result in severe hypokinesis or akinesis (36). Thus, even a small subendocardial AMI will produce a significant abnormality in RF. Furthermore, this wall-thickening abnormality is persistent, and is detectable irrespective of the time delay between the event and imaging. This explains the presence of abnormal RF in 97% of patients who developed an early AMI in our study, even if imaging was performed hours after CP symptoms had resolved (37).

In unstable angina, the presence of a subocclusive coronary stenosis/thrombus and severe decreases in perfusion pressure should also be associated with RF and MP abnormalities (38, 39). In transient ischemia, however, RF and MP abnormalities may resolve especially if there is a significant delay between the ischemic episode and imaging. The negative predictive value of MCE was thus evaluated for patients presenting at different time points after resolution of their symptoms, and was found to remain extremely high (94% for the development of any cardiac event) even up to 12 hrs after CP had resolved (37). Therefore, despite spontaneous reperfusion and restoration of normal antegrade flow, myocardial stunning persists for many hours in patients who have suffered significant ischemia.

Although patients presenting with CP who have abnormal RF and MP on MCE have a significantly higher risk of early and late adverse cardiac events, we found the positive predictive value of MCE to be only 34%, because patients with prior myocardial infarction were not excluded and the presence of existing abnormalities confounded the evaluation of these patients. Comparisons with a previous MCE study would allow the detection of new RF and MP abnormalities, but many patients do not have prior studies for comparison. In another study, where patients with a history of AMI were excluded, the presence of MP defects on MCE was associated with a positive predictive value of 98% for detection of an acute coronary syndrome (40). In clinical practice, however, it is not possible to exclude a significant proportion of patients in this way. Are there methods to...
determine whether a patient’s CP reflects recent ischemia?

Future Directions—Molecular Imaging

The ability of contrast ultrasound (CU) to detect and image specific molecular events within the circulation is an area of vigorous research at this time. For this application, contrast agents have been developed that are targeted to the intravascular molecular and cellular events that occur during inflammation, angiogenesis, and thrombosis. The field of ultrasound molecular imaging is now beginning to transition from development and feasibility testing to the next phase, in which the relative utility of targeted imaging is tested in animal models of disease. One example is the ability to detect acute inflammation that develops in a myriad of cardiovascular diseases, including ischemia-reperfusion injury, which is particularly germane to the present review.

The migration of leukocytes from the circulation into tissue is a key component of the inflammatory response. Initial leukocyte capture and rolling are mediated by selectins (P- and E-selectin) on the endothelial surface that interact with carbohydrate counterligands expressed on leukocytes (L-selectin) (41). The inflammatory markers CD40 ligand, P-selectin, and myeloperoxidase have been shown to increase significantly even after brief periods of ischemia, such as during angioplasty (42). As the leukocytes roll on the venular surface, they become progressively activated and express integrins that interact with intercellular- or vascular-cell adhesion molecules (ICAM-1 and VCAM-1) on the endothelial surface (43). These interactions result in firm adherence of the leukocyte, and eventual trans-migration of the cells via endothelial clefts into the interstitial space according to chemokine signals (43).

The detection of inflammation using CU can be accomplished by changing the rheology of microbubbles in the circulation. Similar to leukocytes, freely flowing microbubbles can accumulate at sites of inflammation by attaching to upregulated molecules there. Initial methods for imaging inflammation by microbubble retention in inflamed tissue relied on nonspecific interactions between microbubbles and activated leukocytes (Fig. 10) (44). The denatured albumin shell of Optison could attach to leukocytes via the β2-integrin Mac-1, and lipid microbubbles such as Definity attached by opsonization with serum complement (44). More recently, specific “targeted” microbubbles have been developed with more robust attachment to activated leukocytes, or even the endothelial surface itself. Strategies that have been used include the addition of phosphatidylserine to the lipid shell to increase complement deposition (45), or the conjugation of specific ligands (such as monoclonal antibodies or peptides) to the microbubble surface (46, 47). For the latter, chemical spacers like polyethylene glycol can be used to project the ligand away from the microbubble surface to decrease steric hindrance and further increase microbubble affinity for the disease-related antigen being targeted (Fig. 10). This technique has been used to specifically image the molecular mediators of leukocyte recruitment such as the selectins, ICAM-1, VCAM-1, and MadCAM-1.

Apart from developing microbubbles that can be retained in sufficient number for detecting the severity and spatial extent of the inflammatory response, other unique challenges exist for imaging targeted microbubbles. First, microbubbles that are attached to cells, or even those that have been phagocytosed by leukocytes, must continue to generate strong acoustic signals for clinical imaging. As noted above, the strength of backscatter signals derived from microbubbles is partly related to oscillations of microbubbles in a sound field. In vitro studies have demonstrated that microbubble oscillations are damped by the increased density of cellular cytoplasm after phagocytosis, and that the oscillations occur at a higher frequency than those of free microbubbles, but acoustic signals are still produced similar to those of free microbubbles, albeit with a frequency...
shift (1). Importantly, the shifted acoustic signals are still within the range of current broadband transducers used clinically. Second, only a small percentage of microbubbles are retained in tissue, and their signal must be differentiated from both tissue clutter and the signal from circulating microbubbles. Because freely circulating microbubbles are cleared from the blood after 5 min to 10 min, molecular imaging with CU has been modified compared with the usual protocol for perfusion imaging (45, 47). After administration of microbubbles, time is given for the targeted microbubbles to accrue in tissue that manifests the antigen of interest, and also for freely circulating microbubbles to clear. Subsequently, imaging is performed to detect only microbubbles retained in the circulation or within leukocytes, using imaging algorithms designed to detect nonlinear signals that emanate only from microbubbles rather than from tissue.

An image using the approach outlined above is shown in Figure 11. In this animal-experimental study, either the left anterior descending or circumflex coronary artery was occluded for 90 mins, followed by reperfusion. CU was performed at various time points after reperfusion using $1 \times 10^{10}$ phosphatidylycerine-lipid-shelled microbubbles targeted to activated leukocytes, followed by imaging 15 mins after their intravenous injection. Images in Figure 11 were obtained using CU, $^{99m}$Tc-RP517 (a radionuclide that binds to the leukotriene $B_4$ receptor of activated neutrophils), and a triphenyl tetrazolium chloride–stained slice of myocardium (to delineate infarction) from an animal after occlusion of the circumflex coronary artery and 60 mins of reperfusion. The short-axis back-ground-subtracted color-coded CU image (Fig. 11A) demonstrates multiple findings. There is a small nonenhanced sub-endocardial rim of myocardium in the lateral wall that reflects the no-reflow zone within the infarct bed—an area characterized by myocellular necrosis and capillary damage that excludes microbubbles (arrow). The no-reflow zone on the CU image is surrounded by an area of contrast enhancement from retained targeted microbubbles. The location and spatial extent of inflammation on CU matches closely to that using $^{99m}$Tc-RP517 (Fig. 11B). The entire infarct bed is delineated with the triphenyl tetrazolium chloride (Fig. 11C). The extent of inflammation on CU was evaluated at multiple time points after reperfusion (45), and was found to slowly decrease over time. Even up to 120 mins after reperfusion, such CU images of “ischemic memory” will be able to define the presence of recent ischemia, but significant delays between the event and imaging may limit the sensitivity of CU. Such issues still require further study.

Although leukocyte targeting may be useful for detecting severe ischemic injury and recent necrosis, it is unlikely to be the sole strategy for early detection of ischemia in patients. The diagnosis of recent ischemia without necrosis must rely instead on the detection of molecular beacons of more subtle injury. The detection of selectins is a focus of recent investigation due to its rapid translocation to the endothelial cell surface in response to even mild ischemic events that do not necessarily progress to a stage of leukocyte accumulation in tissue. A method for molecular imaging of recent ischemia may not only be important for early diagnosis, but also for guiding therapy in those patients with known complex coronary artery disease in whom the size of the ischemic territory may help dictate whether conservative or aggressive therapy is appropriate. These hypotheses require validation in the future.

**SUMMARY**

MCE has revolutionized the field of echocardiography by eliminating problems caused by an inadequate acoustic window, allowing the assessment of LV systolic function in virtually all patients. The free-flowing intravascular microbubble tracers also provide us with the ability to assess myocardial perfusion, not merely spatially, but also temporally.
through the assessment of MBF. In the future, modifications of the shell surface of microbubbles may allow them to demonstrate changes in the molecular milieu of the microcirculation. This multifaceted technology promises to be of increasing clinical utility not only for the heart, but for any organ accessible to ultrasound.

REFERENCES


