EXPERIMENTAL STUDIES

Detection of Coronary Stenosis and Myocardial Viability Using a Single Intravenous Bolus Injection of BR14

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OBJECTIVES

The aim of the study was to determine whether coronary stenosis can be detected and myocardial viability assessed after myocardial infarction from a single venous bolus injection of BR14, a new ultrasound contrast agent.

BACKGROUND

BR14 is an ultrasound contrast agent that, like 201Tl, demonstrates redistribution. Whether this principle can be used to determine myocardial viability is not known.

METHODS

Non-critical (n = 6) or flow-limiting (n = 4) stenoses were placed on coronary arteries of 10 open-chest, dogs, which then underwent 2 h of coronary occlusion followed by reperfusion through the stenosis. Hyperemia was induced to create flow mismatch in the dogs with non-critical stenosis. Hyperemia was not induced in dogs with reduced resting coronary blood flow. All dogs were given 2 ml of BR14 as a bolus injection and serial images were obtained. Myocardial blood flow (MBF) was measured using radiolabeled microspheres. At the end of the experiment, tissue staining was performed to determine infarct size and topography.

RESULTS

Initial images demonstrated flow mismatch between the normal bed and that subtended by the stenosis (during hyperemia in dogs without critical stenosis and during rest in those with reduced resting MBF). The perfusion defect size correlated well with radiolabeled microsphere-derived hypoperfused zone (r = 0.89). Regions within the hypoperfused zone that had not undergone necrosis showed redistribution, whereas the necrotic regions showed a persistent defect, the size of which correlated well with infarct size (r = 0.80). Because of its ability to redistribute, BR14 can define regions of relative hypoperfusion and also discriminate between infarcted and viable tissue within the hypoperfused zone after a single venous injection. This property lends itself to assessing myocardial perfusion during exercise stress.

CONCLUSIONS

We have recently reported that BR14, a novel third-generation ultrasound contrast agent, demonstrates myocardial redistribution after a single intravenous bolus injection (1). This property is based on the initial flow-related distribution of the agent to myocardial beds with transient capillary retention of the agent. The washout of the bubbles is faster from high flow than from low flow regions, which continue to accumulate microbubbles on repeated passes, resulting in equalization of acoustic signal from all beds over time. The kinetics of this agent are therefore very similar to those of 201Tl (2), although the myocardial effect is much shorter (minutes instead of hours). Because ultrasound data acquisition is much more rapid than nuclear data acquisition using standard gamma cameras, the myocardial kinetics of BR14 can be accurately captured with ultrasound despite BR14's short myocardial effect.

We therefore hypothesized that multiple images acquired over time following a single bolus injection of BR14 could be used to detect not only relative hypoperfusion distal to a stenosis, but also the presence and extent of viable tissue in the hypoperfused zone. To test this hypothesis, we used an open-chest canine model of partial to complete infarction distal to either a non-critical or a flow-limiting coronary stenosis.

METHODS

Animal preparation. The study was approved by the Animal Research Committee at the University of Virginia and conformed to the American Heart Association Guidelines for the Use of Animals in Research. Ten adult mongrel dogs were used for the study. They were anesthetized with 30 mg·kg⁻¹ of sodium pentobarbital (Abbott Laboratories, Chicago, Illinois), intubated and ventilated with room air with a respiratory pump (model 607, Harvard Apparatus, Natick, Massachusetts). Anesthesia was maintained during
the experiment with additional sodium pentobarbital as required. Catheters were inserted into both femoral arteries for withdrawal of duplicate reference samples during radio
dabeled microsphere injections, and into both femoral veins for administration of drugs, fluids and microbubbles.

A left lateral thoracotomy was performed and the heart was suspended in a pericardial cradle. Catheters were placed in the aortic root as well as the right and left atria for pressure measurements. The left atrial catheter was also used for the injection of radiolabeled microspheres. The proximal sections of the left anterior descending coronary artery (LAD) and left circumflex coronary artery (LCx) were dissected from the surrounding tissue. Ultrasonic flow
probes (series SC, Transonics, Mt. Pleasant, Middlesex, UK) were placed on both coronary arteries and connected to a digital flow meter (model T206, Transonics) to monitor epicardial coronary blood flow (CBF). A custom-designed screw-occluder was also placed around coronary arteries to enable the production of coronary stenoses of varying severities. A 20 g catheter was introduced into a distal side branch of the coronary artery for the measurement of coronary pressure distal to the stenosis in order to gauge its severity.

All catheters were connected to fluid pressure transducers, which, along with the flow meters, were interfaced with a multi-channel recorder (model ES 2000, Gould Electronics, Eastlake, Ohio). Mean pressures and epicardial CBF were measured digitally and the signals were displayed online using Labtech Notebook (Laboratory Technologies, Bethesda, Maryland).

Myocardial contrast echocardiography. Power pulse-inversion imaging was performed with a HDI 5000 system (Phillips-ATL, Bothell, Washington). The ultrasound transducer (transmit frequency of 1.6 and receive frequency of 3.3 MHz) was fixed in position in a saline bath, which served as an acoustic interface between the transducer and the heart. Imaging was performed at the left ventricular (LV) mid-papillary muscle short-axis level caudal to the occluder. The transmit power, image depth, and color gains were optimized at the beginning of each experiment and held constant throughout. The focal plane was placed at the level of the LV posterior wall. A pulse repetition frequency of 2,500 Hz and a mechanical index of 0.1 were used. Only end-systolic images (gated to the T wave on the electrocardiogram) were acquired and stored digitally using HDI lab. This system allows direct measurement of acoustic intensity (AI) in linear units before log compression and post
processing.

BR14 (Bracco Diagnostics, Geneva, Switzerland) was administered as a controlled 2 ml bolus over 30 s using a power injector (Medrad, Pittsburgh, Pennsylvania). This is a third-generation ultrasound contrast agent consisting of perfluorocarbon-containing microbubbles stabilized by a phospholipid monolayer. The mean diameter of the micro
bubbles is 2.5 to 3.0 μm and their mean concentration is 2–5×10⁹/ml⁻¹ (3). Every end-systolic frame was captured for the first 30 s after the injection. The pulsing interval was then increased to every 10 cardiac cycles for 1 min and then every 20 cardiac cycles for the next 10 min in order to minimize microbubble destruction. After alignment of all images from a single injection sequence (4), regions of interest were placed over different portions of the LAD and LCx beds for measurement of AI, which was then plotted against time. The resultant curves from normal, infarction and viable tissue were compared.

Radiolabeled microsphere analysis. Myocardial blood flow (MBF) was measured using left atrial injections of ~2 × 10⁶–11 μm radiolabeled microspheres (Dupont Medical Products) suspended in 4 ml of 0.9% saline and 0.01% Tween-80. Duplicate arterial reference blood sam
ples were collected from the femoral arteries. At the end of the experiment, the post mortem LV short-axis slice corre
sponding to the myocardial contrast echocardiography (MCE) image was cut into 16 wedge-shaped pieces, and each piece was further divided into epicardial, mid- and endocardial portions. The tissue and blood reference samples were counted in a gamma well scintillation camera with a multi-channel analyzer (model 1282, LKB Wallace, Wellsley, Massachusetts), and corrections were made for activity spilling from one energy window to another with custom

designed software.

Myocardial blood flow to each myocardial segment was calculated from the equation \( Q_m = \frac{(C_m Q_t)}{C_r} \), where \( Q_m \) is blood flow to the myocardial segment (ml/min⁻¹), \( C_m \) is tissue counts, \( Q_t \) is the rate of arterial blood sample withdrawal (ml/min⁻¹), and \( C_r \) is arterial reference sample counts (5). Transmural MBF (ml/min/g) to each of the 16 wedge-shaped pieces was calculated as the quotient of the summed flows to the individual segments within that piece and their combined weight. Mean MBF to each region of interest was then calculated by averaging the transmural MBF in that region, which was defined as will be described.

Myocardial blood flow in each segment of the LV was also represented with a parametric image to display the magnitude of MBF. This custom-designed program uses colors ranging from orange-brown (low MBF) to white-yellow (high MBF). All values are normalized to the highest MBF within the LV short-axis slice. Myocardial blood flows between adjacent segments are averaged to allow a smoother transition of color. The regions depicting low
MBF were tested by planimeter and expressed as a percentage of the LV short-axis area.

Infarct size determination. The LV short-axis slice corresponding to the MCE image was immersed in a solution of 1.3% 2,3,5-TTC (Sigma, St. Louis, Missouri) and 0.2 M Sörensen’s buffer (KH₂PO₄ and K₂HPO₄ in distilled water, pH 7.4) at 37°C for 20 min followed by fixation in 10% formalin (6). Digital photographic images of these slices were transferred to computer and the infarct region was tested by planimeter and expressed as a percentage of the LV short-axis area.

Experimental protocol. The purpose of the study was to determine if MBF mismatch could be determined from initial images following a single injection of BR14 and whether late images would provide information on myocardial viability. Accordingly, a non-critical stenosis that did not affect resting CBF was placed on the LAD (n = 4) or LCx (n = 2) in six Group I dogs. In four dogs (Group II), a critical stenosis that reduced both CBF and regional function was placed on either the LAD (n = 2) or LCx (n = 2). The artery with stenosis was then occluded for 2 to 3 h to produce necrosis of varying degrees within the myocardium distal to the stenosis. The occlusion was then reversed, but the stenosis was left unaltered. In this manner, a model of coronary stenosis and partial infarction distal to the stenosis was created to mimic a common clinical setting.

In Group I dogs, maximal coronary hyperemia was induced with a continuous infusion of 1 µg/kg⁻¹/min⁻¹ of the selective adenosine A₂a receptor agonist 2-cyclohexylmethyl-idenehydrazino-adenosine (WRC-0470, Discovery Therapeutics, Richmond, Virginia) in order to produce MBF disparity between the stenosed and normal beds. Because this disparity was already present in the Group II dogs, hyperemia was not induced in them. Radiolabeled microspheres were injected, following which a bolus of BR14 was administered and MCE was performed in order to obtain early and late images. Immediately after completion of imaging, the dog was sacrificed and the heart slice corresponding to the MCE image was stained with TTC, photographed and processed for radiolabeled microsphere MBF analysis.

Statistical methods. Data are expressed as mean ± 1 standard deviation. Differences between hypoperfused and normal myocardium in the initial images as well as between viable and infarcted myocardium in the delayed images were assessed using Student’s paired t test. Correlations were calculated using least-fit regression analyses. Differences were considered significant at p < 0.05 (two-sided).

RESULTS

Group I dogs. Figure 1 illustrates images from one Group I dog. During hyperemia, a large zone of hypoperfusion was noted in the bed supplied by the non-critical stenosis (Fig. 1B) that corresponded to the region with relatively lower radiolabeled-microsphere derived MBF (Fig. 1B). Within 10 min, however, this hypoperfused zone was significantly smaller (Fig. 1C) and corresponded to the infarct size on TTC staining (Fig. 1D). Figure 2 depicts time-versus-AI plots from different myocardial regions. The non-necrotic region within the initially hypoperfused zone showed an
increase in AI to the level seen in the normal myocardium, whereas the necrotic area showed no change in AI during the entire imaging sequence. Similar results were found in all Group I dogs.

Group II dogs. This group of dogs did not undergo hyperemia because resting MBF was already reduced because of a critical stenosis. Figure 3 depicts images from one Group II dog. A large hypoperfused zone was seen distal to the stenosis initially after injection of BR14 (Fig. 3B) corresponding to the zone of reduced resting MBF as measured by radiolabeled microspheres (Fig. 3A). The normal bed showed good perfusion consistent with the

Figure 2. Changes in acoustic intensity (AI) values from the left anterior descending (LAD) and left circumflex coronary artery (LCx) beds (denoted on the images in Fig. 1) following the injection of BR14 during maximal hyperemia. The LCx has a residual non-critical stenosis, whereas the LAD bed is normal. Although a flow mismatch is seen early after injection, AI in all viable tissue is similar at a later point in time, indicating redistribution. Where infarction had occurred a persistent perfusion defect remained. See text for details.

Figure 3. Example from a Group II dog that had reduced resting myocardial blood flow (MBF). (A) Parametric image obtained from microsphere derived-MBF indicating an ischemic LCx territory. The MCE image taken early after injection matches this image (B), but the late MCE image (C) matches the infarction (D). See text for details. The preparation of this animal was the same as in Figure 1 except that hyperemia was not used. Other abbreviations as in Figure 1.
microsphere data. Similar to the Group I dogs, however, the region within the hypoperfused bed that had not undergone necrosis showed normalization of AI on late imaging (Fig. 3C). Only the necrotic regions (confirmed on TTC staining) continued to show persistent hypoperfusion (Fig. 3D). Figure 4 depicts time-versus-AI plots from different myocardial regions. Again, the necrotic region showed no change in AI over time, whereas the viable region showed redistribution. All Group II dogs showed similar results.

Combined results. Figure 5 shows the relation between the size of the hypoperfused bed on MCE during the initial imaging period and the radiolabeled-microsphere derived extent of relative hypoperfusion. As expected, a close relation was observed. Figure 6 depicts the relation between the extent of hypoperfused myocardium during late imaging and the infarct size measured by TTC staining. Once again, a good correlation is noted between the two. Thus, with a single injection of BR14, both the region of relative hypoperfusion (potentially ischemic in Group I and actually ischemic in Group II dogs) as well as the infarct size could be measured during MCE. Regions with relative hypoperfusion that had not undergone necrosis demonstrated redistribution. The AI ratios between the hypoperfused and normal beds within necrotic and viable tissue over time are

![Figure 4.](image)

**Figure 4.** Changes in AI values from the LAD and LCx beds (denoted on the images in Figure 3) following the injection of BR14. The LCx has a residual critical stenosis, whereas the LAD bed is normal. Although a flow mismatch is seen early after injection, AI in all viable tissue is similar at a later point in time, indicating redistribution. Where infarction had occurred, a persistent perfusion defect remained. See text for details. Abbreviations as in Figure 2.

![Figure 5.](image)

**Figure 5.** Correlation between radiolabeled-microsphere derived hypoperfused territory and myocardial contrast echocardiography-derived risk area and in all 10 dogs. See text for details. LV = left ventricular; MCE = myocardial contrast echocardiography.
shown in Table 1. These ratios were significantly different between the infarcted and viable myocardium. In particular, viable myocardium had the same VI as the normal myocardium at late imaging.

**Observer variability.** The correlation coefficients between two sets of observations (n = 12) performed by the same observer (intraobserver) and two sets performed by two separate observers (interobserver) were 0.97 (SEE = 8%) and 0.96 (SEE = 9%) respectively.

**DISCUSSION**

In this study we have shown that a single injection of BR14 can be used to detect relative hypoperfusion distal to a stenosis as well as the extent of viable and/or necrotic tissue within the hypoperfused zone. The discrimination between necrotic and viable tissue is based on the ability of BR14 to redistribute to viable regions (1). These characteristics are very similar to those of $^{201}$Tl (2).

**Assessment of viability using radioisotopes.** When a defect is seen during peak stress on nuclear imaging using single-photon tracers, it implies the presence of hypoperfusion. Whether this hypoperfusion is fixed (i.e., also present at rest, in which case it represents necrotic tissue) or reversible (i.e., no longer present at rest, in which case it represents viable tissue) can be determined with $^{99m}$Tc agents using a second injection at rest (7). Because, unlike most $^{99m}$Tc agents (8,9), $^{201}$Tl demonstrates redistribution (2), a second injection is generally not required to discriminate between infarcted and viable myocardium, although some centers use $^{201}$Tl reinjection to improve count statistics.

This approach has been used in nuclear cardiology because traditionally wall thickening could not be assessed using myocardial perfusion tracers; only relative myocardial perfusion could be measured. In the case of echocardiography, wall thickening can be easily assessed. In this regard, the approach is more physiological and clinically relevant because viability is an issue only when significant regional dysfunction is present at rest. Adequate myocardial enhancement on MCE at rest in a region with reduced function implies the presence of viable myocardium.

**Assessment of viability by MCE.** With most second-generation contrast agents, viability can be easily assessed by the presence of normal or near-normal myocardial enhancement in dysfunctional regions with adequate MBF (stunned myocardium). In dysfunctional regions with reduced MBF (hibernating myocardium), however, the interval between microbubble destruction and imaging needs to be prolonged (10). This approach can be technically challenging in the clinical setting. Our results indicate that with BR14, delayed imaging can answer the question without need for prolonging the pulsing interval. This property of BR14 could make it easier to detect viable myocardium, particularly in low flow regions such as in our Group II dogs.

Another advantage of echocardiography over nuclear
cardiology is the superior spatial resolution allowing assessment of the transmural distribution of myocardial perfusion. As seen from Figures 1 and 3, the perfusion defect on late imaging is similar in topography to the infarct. This property could assist in determining the effect of infarction on recovery of resting function as well as LV remodeling (11). It could thus assist in the proper selection of patients who should undergo vigorous therapy to prevent or attenuate LV remodeling.

The traditional echocardiographic approach for assessment of myocardial viability has been the use of dobutamine (12,13). Although this technique has been clinically very useful, it entails time and unpleasant, if not serious, side effects (14). Our results indicate that a single injection of BR14 can provide an accurate assessment of myocardial viability at rest without recourse to drugs. Furthermore, a direct assessment of the spatial distribution of necrosis may offer more insights toward individual patient management than simply the ability of the myocardium to respond to catecholamines (10,15).

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