Technetium-99m-Nitroimadazole Uptake in a Swine Model of Demand Ischemia

Lynne L. Johnson, Lorraine Schofield, Patricia Mastrofrancesco, Tammy Donahay and Lorraine Nott
Division of Cardiology, Rhode Island Hospital, Providence, Rhode Island

Nitroheterocycles are electron affinic, lipophilic compounds that are retained in hypoxic tissue. This study was designed to test the hypothesis that ^{99m}Tc-5-oxa-amine-oxime nitroimadazole (BMS-194796) is retained in ischemic myocardial tissue in a swine model of demand ischemia and that the retained tracer can be imaged in vivo. **Methods:** Eighteen domestic swine were anesthetized, intubated and instrumented, including placement of a stenois (80% narrowing) mounted on a catheter into the left anterior descending (LAD) coronary artery. Twelve experiments had complete sets of data for analysis. Each animal was paced at about 200 bpm for 4 min, and 28 mCi of ^{99m}Tc BMS-194796 were injected during the last minute of pacing. Dynamic planar imaging was started after pacing and completed at 2.5 hr. In the last 8 experiments, SPECT imaging was performed after planar imaging and completed 3.5 hr after injection. Hemodynamic measurements were made continuously. Blood flow by microspheres and myocardial lactate extraction were measured at control, during pacing and after 2 hr of recovery. The animals were then killed; the risk region was delineated and the hearts were removed, sliced, imaged and stained with triphenyl tetrazolium chloride. **Results:** Nine of the 12 animals became ischemic (net lactate production) during pacing; 3 did not. None of the 3 nonischemic experiments showed focal uptake on ex vivo or in vivo imaging. All 9 of the ischemic experiments showed focal BMS uptake in the risk region on ex vivo imaged slices; 6 of 9 had uptake in the risk region on in vivo imaging; and 4 of these 6 had small scattered areas of subendocardial necrosis in the risk region on triphenyl tetrazolium chloride staining. Four animals had small infarcts in the distribution of proximal LAD branch vessels occluded by the stenosis catheter. All animals with branch vessel infarcts had positive in vivo images. Overall, 8 of 9 ischemic experiments had positive in vivo images. **Conclusion:** These data support the conclusion that focal myocardial retention of BMS-194796 can be visualized on in vivo imaging in closed chest large animal model after intravenous injection.

Key Words: nitroheterocycles; demand ischemia; swine

J Nucl Med 1998; 39:1468–1475

Single-photon imaging agents clinically available provide only indirect measures of ischemia. Tracers such as ^{201}Tl or ^{99m}Tc-sestamibi are taken up by viable, metabolically functioning myocardial cells in the distribution of blood flow, but scan defects in addition to true ischemic defects may represent flow heterogeneity in the absence of ischemia or may be due to myocardial infarction or scar. Other tracers such as ^{99m}Tc-pyrophosphate or ^{111}In-antimyosin are avidly taken up by necrotic myocytes. An agent specific for viable but ischemic myocardium is not available clinically.

Nitroheterocycles are a class of compounds that are electron affinic and lipophilic, enabling them to diffuse readily across cell membranes (*1*). These compounds are not new and have very diverse applications, including use as antibiotics and radiation sensitizers (*2*). A method has been developed to complex ^{99m}Tc through a ligand to a nitroheterocycle moiety, leaving a reactive site for intracellular binding (*3*). Kinetic studies of the first ^{99m}Tc-labeled nitroimidazole compound were developed; BMS-181321 showed retention of the compound in myocardial tissue with low oxygen levels (*4–7*). Although tissue hypoxia can occur in situations other than inadequate or insufficient flow, such as reduced hemoglobin or hemoglobin O_2 binding or in states with reduced blood O_2 levels, usually tissue hypoxia is the result of reduced blood flow. However, focal uptake of BMS on in vivo imaging in either infarct or demand ischemia could not be demonstrated due to poor target-to-background count ratios attributed to relatively slow washout from normoxic tissue and/or upscatter from hepatic activity (*8,9*). One study of low flow ischemia did show focal uptake on tomographic imaging when severely reduced tissue oxygen levels were achieved (*10*). In these animal models of infarction, nitroimidazole uptake was demonstrated at the infarct border zones on ex vivo imaging.

To hasten clearance from normoxic tissue, radiochemists altered the chemical structure of BMS-181321, and initial results using this new compound in an open-chest dog model of severe low flow ischemia showed improved target-to-background ratios (*11*). The previous work evaluating radiolabeled nitroheterocycles has been performed using either low flow ischemia or infarct models. The other common cause for ischemia in patients is demand ischemia. Therefore, this study was designed to test the following hypotheses: ^{99m}Tc BMS-194796 is retained in ischemic, noninfarcted tissue during demand ischemia, and myocardial uptake can be documented by in vivo gamma camera imaging.

MATERIALS AND METHODS

Animal Preparation and Instrumentation

This study was performed within the guidelines specified by the National Institutes of Health for care and use of laboratory animals and with the approval of the Rhode Island Hospital Animal Care Committee. Farm-bred omestic swine were fasted overnight and immobilized with intramuscular xylazine and ketamine. After immobilization, an ear vein was cannulated with an indwelling catheter, and a deeper level of anesthesia was attained with 2.5% intravenous sodium pentothal. Animals were then intubated and anesthesized with isoflurane and nitrous oxide (60:40 mixture with oxygen). Ventilation was maintained throughout instrumentation with a volume-cycled respirator through which supplemental oxygen was given at 2–3 liters/min with room air and anesthetic gases. Arterial blood gases were obtained frequently to maintain constant pH, pO_2 and pCO_2. Heparin was administered (225 U/kg), and heparization was maintained by regular bolus dose injections.

Surgical cutdowns were performed on the neck to expose the right internal carotid artery, right internal jugular vein and the right external jugular vein. Cutdown incisions were also made on both groins to expose the right and left femoral arteries and veins. A #7F Eppendorf catheter was advanced by fluoroscopic control from the right femoral artery into the left ventricle and retrograde across the mitral valve into the left atrium. This catheter was used to administer colored microspheres for measurement of regional

Received Aug. 6, 1997; accepted Nov. 21, 1997.
For correspondence or reprints contact: Lynne L. Johnson, MD, RIH, Main Bldg., Rm. 208, 593 Eddy St., Providence, RI 02903.

myocardial blood flow and to measure left atrium pressures. A #8F double lumen catheter was introduced through the left femoral artery into the thoracic aorta just below the origin of the subclavian artery to monitor blood pressure and arterial blood gases and for withdrawal of microsphere reference blood samples. Two additional venous access lines were established for fluid administration and radiotracer injection. A #8F guiding catheter was placed in the right internal jugular and passed into the coronary sinus and anterior great coronary vein. A 3F catheter was then guided into the interventricular vein over a 0.014 angioplasty wire with a floppy end previously passed through the guiding catheter. A #7F bipolar pacing catheter was inserted via the left internal jugular into the right atrium or coronary sinus for pacing. A plastic stenosis reducing the lumenal diameter by 80% and mounted on the end of percutaneous translumenal coronary angioplasty (PTCA) catheter with balloon cutoff was advanced into the proximal left anterior descending coronary artery (*12*). Isoflurane and nitrous oxide were discontinued, and the animal was allowed to awaken sufficiently to breathe spontaneously and exhibit modest tremulousness. Intravenous pentothal was then begun at 10–40 ml/hr (120 mg/m) to maintain sedation and ensure that the animal was pain-free.

Experimental Protocol

Arterial blood gases were monitored as needed to assure appropriate ventilation. Initial measurement of the following variables were made: aortic and left atrial pressure, atrial pressure, arterial and anterior interventricular vein sampling for lactate, O_2 and pH. The colored microspheres were injected (6 million), and pacing was begun at 170–210 bpm. After 3 min of pacing, repeat hemodynamic and metabolic measurements were made, and a second set of microspheres were injected, followed by bolus injection of ^{99m}Tc BMS. Pacing was then discontinued. Total duration of pacing was 5–7 min. Repeat hemodynamic and metabolic measurements were made 3–4 hr later, and a third set of microspheres was injected. Colored microspheres (15 ± 0.43 μm) used for blood flow determination are cross-linked polystyrene-divinylbenzene microspheres in eight colors: red, blue, orange, green, yellow, coral red, violet and black (E-Z Trac, Los Angeles, CA). These colored microspheres are chemically stable and exhibit no dye leaching even in tissue-exposed strong acid and base solutions (*13,14*).

Tracer Preparation and Imaging Protocol

The nitroimidazole compound was wrapped in foil to exclude light and labeled with ^{99m}Tc by incubation with stannous pentetate. Chromatography was performed using Whatman 31 ED paper prespotted at the origin with acetonitrile and immediately spotted with the ^{99m}Tc BMS-194796. The strip was developed in methylene chloride. The ^{99m}Tc complex moves with the solvent front. Labeling efficiency was >90% for all experiments. The mean administered dose of ^{99m}Tc BMS-194796 was 28 mCi.

For the first two experiments, blood samples were obtained at 1, 5, 10, 30, 60 and 120 min after injection to calculate blood pool clearance. Dynamic left lateral planar imaging was performed for 135 min after injection on an Ohio Nuclear Technicare Sigma 420 camera/CardioMac (NC Systems, Boulder, CO). In the last 8 experiments, after the completion of the planar imaging sequence, the animals underwent tomographic imaging using a dual-detector camera (ADAC Vertex; Milpitas, CA) over 180° arc for 25 sec/stop, 64 projections. The raw data were reconstructed using filtered backprojection with Butterworth filter cutoff frequency 0.45, order 5, and the tranaxial slices were displayed. The mean time from injection to completion of planar imaging was 2 hr, 30 min ± 22 min, and mean time from injection to SPECT imaging was 3 hr, 33 min ± 32 min.

Kill and Pathology

The chest was opened and the coronary artery was ligated at the proximal end of the stenosis. Fluorescein dye was injected into the left atrium to stain the portion of the heart not perfused distal to the stenosis. The animal was then given a large dose of intravenous pentothal, immediately followed by a lethal dose of KCl through the left atrial catheter. The heart was removed, washed and sliced into 1-cm slices. The slices were imaged for 5 min on the camera detector and then immersed in 1.5% triphenyl tetrazolium chloride (TTC) at 37° for 15 min (*15*). The heart slices were then immersion fixed in 10% neutral buffered formalin overnight. Video images of the stained slices of the heart were scanned into the computer. The heart was then prepared for determination of regional myocardial blood flow (*13,14*).

Blood Flow Measurements

Regional myocardial blood flow values were measured using the methods described by Hale et. al. (*13*). Whole weighted tissue

TABLE 1
Imaging Results

Experiment	Vascular bed	In vivo imaging	Ex vivo imaging	TTC staining
Ischemic				
1	RR	Positive planar	Positive	Scattered negative RR
	Infarct	Positive planar	Positive	Negative diagonal infarct
2	RR	Negative planar	Positive	Positive
3	RR	Positive planar	Positive	Scattered negative RR
4	RR	Positive planar and SPECT	Positive	Scattered negative RR
5	RR	Positive planar and SPECT	Positive	Scattered negative RR
6	RR	Negative planar and SPECT	Positive	Positive RR
	Infarct	Positive planar and SPECT	Positive	Negative diagonal infarct
7	RR	Negative planar and SPECT	Positive	Positive RR
	Infarct	Positive planar and SPECT	Positive	Negative diagonal infarct
8	RR	Negative planar, positive SPECT	Positive	Positive RR
9	RR	Positive planar and SPECT	Positive	Positive RR
	Infarct	Positive planar and SPECT	Positive	Negative septal infarct
Nonischemic				
1	RR	Negative planar and SPECT	Very faint diffuse	Positive
2	RR	Negative planar and SPECT	Very faint diffuse	Positive
3	RR	Negative planar and SPECT	Very faint diffuse	Positive

RR = risk region; TTC = tripheny tetrazolium chloride.

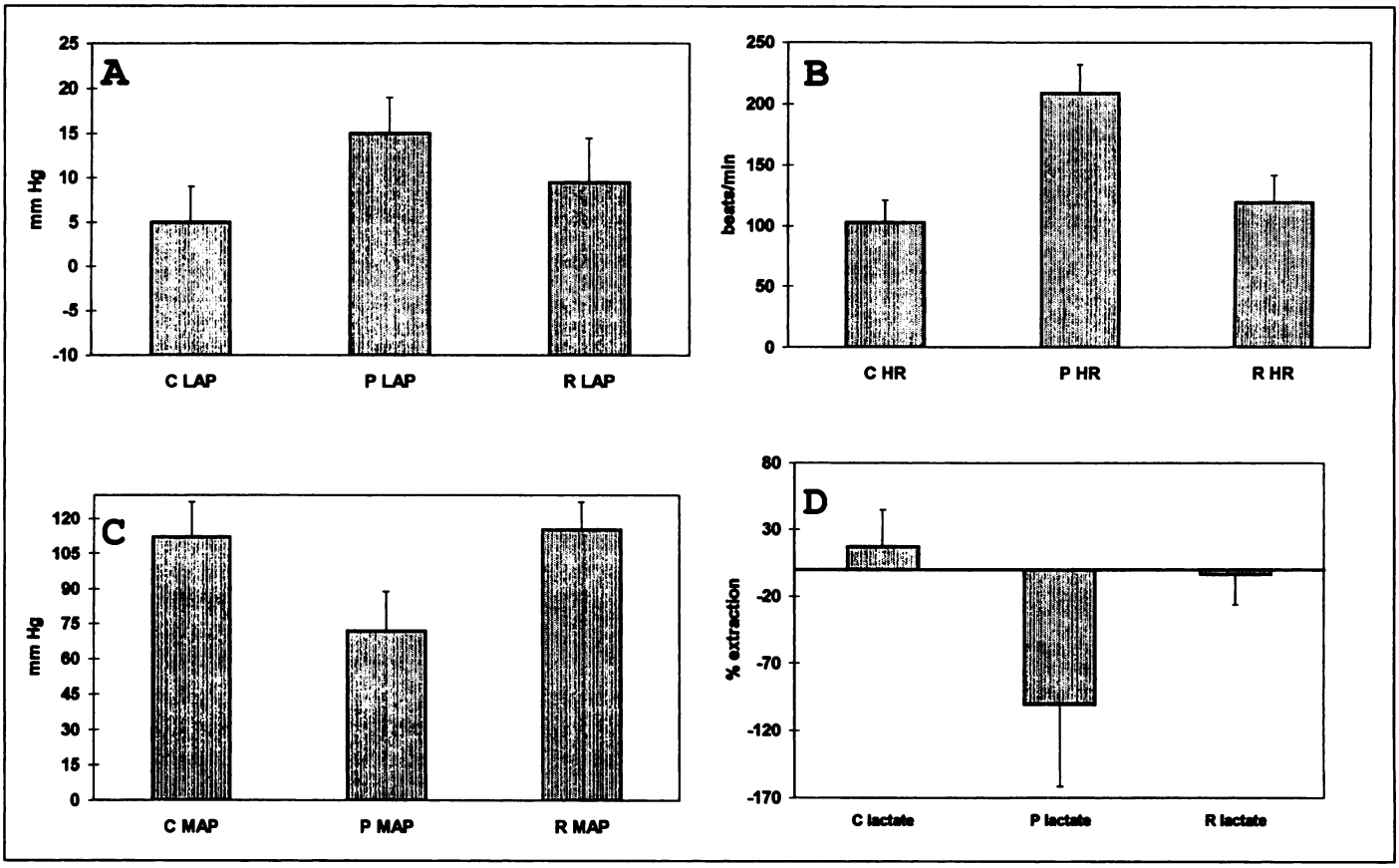

FIGURE 1. Columns represent mean values for hemodynamic and lactate data for the nine ischemic animals; bars represent s.d. C = control; P = pacing; R = recovery; LAP = left atrial pressure (A); HR = heart rate (B); MAP = mean arterial pressure (C). (D) Percentage lactate extraction.

samples of less than 3 g were hydolyzed in 2 *N* NaOH solution overnight and then Tissue Blood Digest Reagent II. Samples were centrifuged and the pellets were washed twice with Microsphere Counting Reagent. The number of colored microspheres in the final tissue and reference blood preparations were counted manually using a Fuchs-Rosenthal hemacytometer counting slide. Either a total of 400 microspheres of each color were counted, or counting was done until the sample was gone.

Tracer Uptake

Tissue uptake of radioactivity was determined both by calculating uptake as percentage of injected dose and by calculating count ratios for the risk region/remote myocardium. The planar image of the cardiac slices imaged ex vivo on the detector face for each experiment was displayed on the computer screen, and regions of interest (ROIs) were drawn around the risk regions, normal regions and infarcted regions (confined to the basal slices). The regions were created, using as guide a drawing of the TTC and fluorescein-stained heart slices, mounted in the identical orientation. Counts in the risk ROIs for all slices were summed for calculation of percentage of injected dose in the risk region, and counts in the infarct regions of interest for all slices comprising the infarct (usually one or two basal slices) were summed. To calculate percentage of injected dose, counts/min in the risk region or infarct region were divided by the product of the injected dose and the camera efficiency. In addition, count ratios were calculated as ratio of counts in the risk region to counts in the normal regions.

Statistical Analysis

The within-group variations for the ischemic group were analyzed using a one-way repeated measures ANOVA. To compare the ischemic and nonischemic groups, an independent-samples Student's t-test was used.

RESULTS

Experiments Performed

A total of 18 experiments were performed. The results of 6 could not be used for the following reasons. Two animals died before completion of the protocol, 2 animals were hemodynamically unstable and had diffuse myocardial ischemia by flows, in 1 experiment there were problems with the microsphere data and in 1 experiment the stenosis catheter dislodged. Based on lactate extraction and reduced endocardial blood flow, three of the animals did not become ischemic, and nine did. Results of the nine successful experiments that became ischemic based on blood flow and lactate measurements are summarized in Tables 1 and Figures 1 and 2.

Hemodynamic and Lactate Data

Hemodynamic and lactate data are summarized in Figure 1. The heart rate went from 103 ± 18 (control) to 209 ± 23 (pacing) and to 119 ± 22 (recovery). Left atrial pressure values went from 5 ± 4 mmHg (control) to 15 ± 4 mmHg (pacing) to 9 ± 5 mmHg (recovery). Repeated-measures p value was <0.001, reflecting a significant rise in left atrial pressure with pacing. Mean arterial pressures went from 112 ± 15 mmHg (control) to 72 ± 17 mmHg (pacing) to 115 ± 12 mmHg (recovery). Repeated-measures p value was <0.001, reflecting a significant fall in mean arterial pressure with pacing. Values for percentage lactate extraction were 17% ± 28% control, −101% ± 61% during pacing and −3% ± 23% at recovery. Repeated-measures p value was <0.001, reflecting the shift from net lactate extraction to net production with pacing.

Blood Flow

Transmural blood flow in the normally perfused myocardium went from 1.33 ± 0.38 (control) cc/g/min to 1.29 ± 0.53

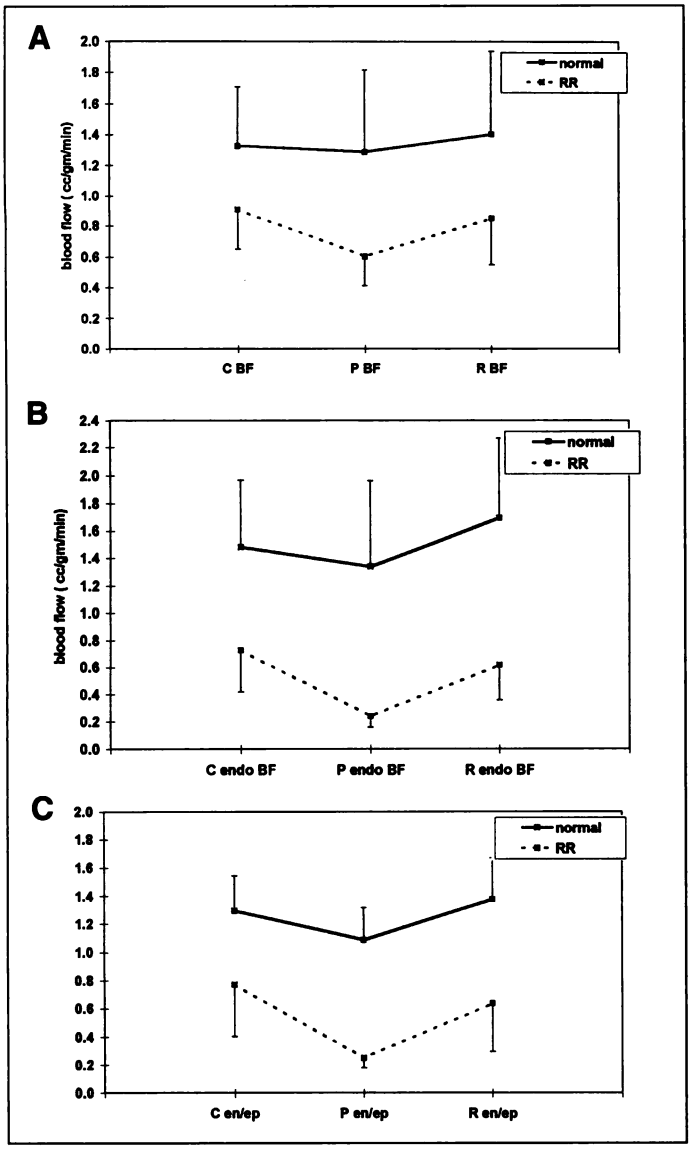

FIGURE 2. Data points represent mean values for myocardial blood flow data for the 9 ischemic animals during control (C), pacing (P) and recovery (R); bars represent s.d. Normal zone is represented by the solid line, and the risk region is represented by the broken line. (A) Transmural blood flow (BF) data. (B) Endocardial blood flow (endo BF) data. (C) Endo-to-epi blood flow ratios. All risk region values are significantly lower than normal region values, and in addition, the pacing values in the risk region are significantly lower than control values in the risk region (see text).

cc/g/min (pacing) to 1.40 ± 0.54 cc/g/min (recovery) (Fig. 2). The repeated-measures p value was 0.78, reflecting the lack of any significant changes in transmural blood flow during the experiment. Transmural blood flow in the risk region went from 0.91 ± 0.26 cc/g/min (control) to 0.60 ± 0.19 cc/g/min (pacing) to 0.85 ± 0.30 cc/g/min (recovery). The repeated-measures p value was <0.05, reflecting a significant fall in transmural blood flow with pacing. Endocardial blood flow in the normally perfused myocardium went from 1.48 ± 0.48 cc/g/min (control) to 1.34 ± 0.62 cc/g/min (pacing) to 1.69 ± 0.58 cc/g/min (recovery), whereas endocardial blood flow in the risk region fell from 0.73 ± 0.31 cc/g/min (control) to 0.24 ± 0.08 cc/g/min (pacing) and rose to 0.62 ± 0.26 cc/g/min (recovery). The repeated-measures p value was <0.001, reflecting the highly significant fall in the endocardial blood flow with pacing. The endocardial-to-epicardial blood flow ratios in the normal region did not change significantly during the experiment, going from 1.29 ± 0.25 (control) to 1.09 ± 0.23 (pacing) and 1.38 ± 0.29 (recovery), whereas in the risk region, the ratios fell from 0.77 ± 0.37 (control) to 0.25 ± 0.07 (pacing) and rose to 0.64 ± 0.34 (recovery). The repeated-measures p value was <0.001, reflecting the significant fall in the endocardial-to-epicardial ratio in the ischemic zone. For all three variables and for all three states (control, pacing and recovery), risk region values were significantly lower than normal region values at the $p < 0.001$ level.

Pathology

The 2 nonischemic animals were TTC-positive in the risk region. Of the 9 ischemic animals, 6 were TTC-positive in the risk region, and 4 showed scattered focal patches of TTC-negative staining in the subendocardium. Of these 4 latter experiments, 2 showed the lowest levels for endocardial blood flow during pacing in the risk region (0.11 and 0.16 ml/g/min), and the third developed VT immediately after the end of pacing, requiring lidocaine, and continued to have a sinus tachycardia throughout recovery period (heart rate = 151). Four of the experiments showed discrete focal areas of TTC-negative staining localized to the distribution of a proximal branch vessel and seen only on the basal slices: diagonal branch (*3*) or the first septal perforator (*1*).

Imaging Data

The rate of tracer clearance from the blood pool was calculated from data acquired from the first 2 experiments. The curves were fit with use of 2 exponentials, and the $T_{1/2}$ for the first component was 1.7 min and for the second $T_{1/2}$ was 57.8 min. In all experiments with both planar and SPECT imaging, focal tracer activity in the heart was easier to identify on the lateral planar projection image than on the standard planar images due to less background myocardial activity on the images acquired 1 hr later.

The imaging results are summarized in Table 1. The 2 experiments without metabolic or blood flow evidence for pacing-induced ischemia showed only very faint patchy myocardial uptake that could only be seen by lowering the upper threshold. Neither of these experiments showed uptake on either planar or SPECT imaging. An example of a negative study is shown in Figures 3 and 4.

Eight of the 9 ischemic experiments showed focal tracer uptake on in vivo imaging. The 1 false-negative experiment did not undergo SPECT imaging. Four of the 9 experiments also had branch vessel infarcts. The branch infarct territories could always be distinguished from the risk region on ex vivo slices because they were very discrete and confined to the basal slices, and in all experiments, the BMS uptake in the region of the small branch vessel infarcts was more intense than uptake in the risk region. All of the ischemic experiments showed focal BMS uptake in the risk region on ex vivo imaged slices, whereas on in vivo imaging, BMS uptake could be localized to the risk region in 6 experiments. In 2 of these 6 experiments, tracer could be localized to both the risk region and to an infarct territory with the aid of SPECT (Experiment #9, Table 1) or based on the extent of tracer uptake on the planar images (Experiment #1, Table 1). Four of the 6 experiments with uptake in the risk region on ex vivo imaging showed scattered focal patches of TTC-negative staining in the subendocardium (Figs. 5 and 6). Three of the 4 animals with branch vessel infarcts underwent SPECT imaging, and in 2 of these experiments, focal uptake appeared to be localized only to the branch vessel territory, and uptake in the risk region was not seen (Figs. 7 and 8). One experiment without any infarction was positive on SPECT images but negative on planar images (Experiment #8, Table 1).

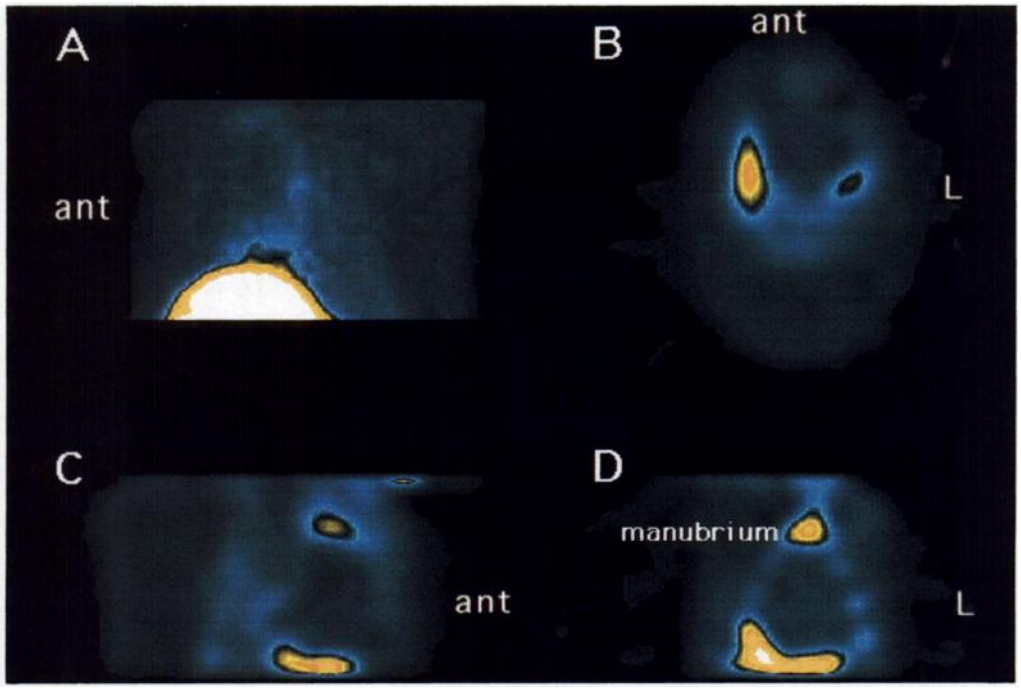

FIGURE 3. Left lateral projection image (A), transverse (B), sagittal (C) and coronal (D) tomographic reconstructed slices. No focal tracer uptake is seen in the region of the heart on tomographic scans.

For the 9 ischemic animals, the mean percentage of injected dose in the risk region was 0.25% ± 0.11% (range 0.12%–0.41%). The mean ratio of counts in the risk region to normal was 2.38 ± 0.61 (range 1.57–3.22). When count ratio for risk region/normal was plotted versus risk region endo-to-epi blood flow ratio, the correlation coefficient was 0.718 ($p < 0.05$) (Fig. 9).

DISCUSSION

Nitroheterocycles are a class of compounds that are electron-affinic and lipophilic, enabling them to diffuse readily across cell membranes. In the presence of low levels of intracellular oxygen, they are unable to diffuse back out of cells (*1*). In an isolated perfused rat heart model, Rumsey et al. (*3*) demonstrated a biphasic rise in tissue levels of BMS-181321, showing

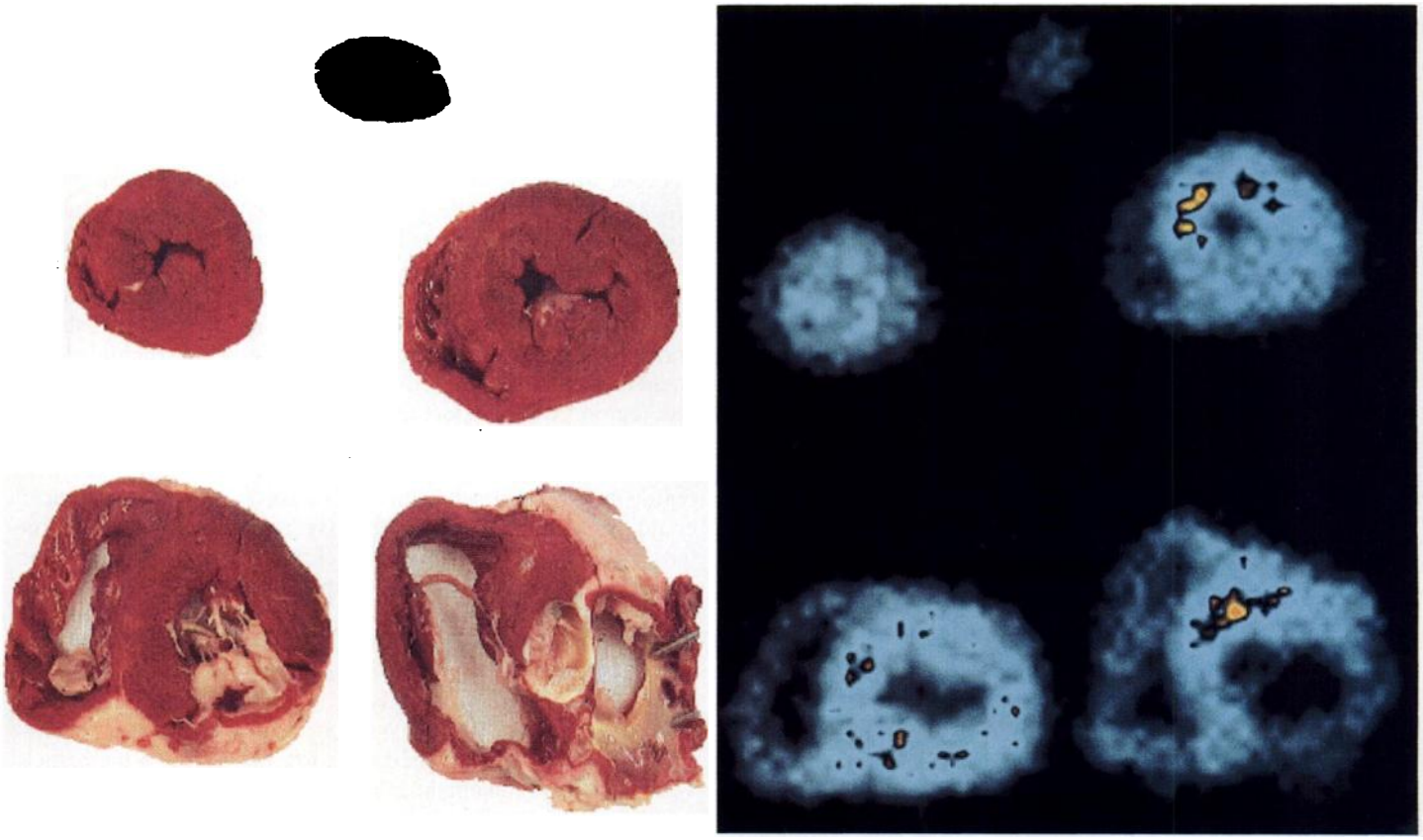

FIGURE 4. Myocardial slices from experiment shown in Figure 3. Triphenyl tetrazolium chloride- (TTC-) stained slices are on the left, and ex vivo-imaged slices are on the right. The TTC staining was positive throughout, and there is minimal tracer uptake on the ex vivo-imaged slices.

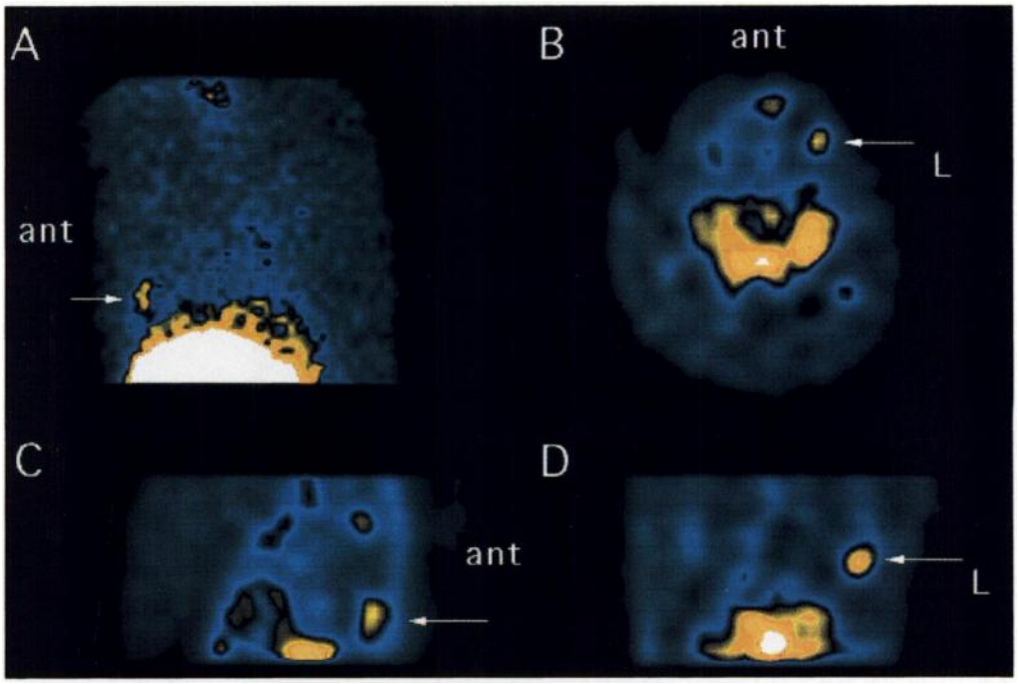

FIGURE 5. Left lateral projection image (A), transverse (B), sagittal (C) and coronal (D) tomographic reconstructed slices. Arrow points to BMS uptake seen on left lateral projection image and on tomographic slices and corresponds to anterior wall of the left ventricle (risk region).

a rapid initial rise followed by a slower rise to peak in both normoxic and hypoxic myocardium. Washout was also biphasic, with an early rapid phase followed by a late slow phase. For hypoxic compared to normoxic myocardium, activity levels were greater during and after infusion. The association of BMS-181321 with cardiac myocytes was greatest when intracellular oxygen levels were lowest. In the presence of oxygen, there was no association between BMS-181321 uptake and intracellular energy level, thereby eliminating the possibility that changes in cellular energy levels rather than oxygen levels were responsible for retention in hypoxic cells.

In a subsequent study, ^{99m}Tc BMS-181321 was injected into spontaneously hypertensive rats after middle cerebral artery occlusion, and uptake was seen in the ischemic tissue at risk, but not in the infarcted tissue (*16*). Kinetic analysis of ^{99m}Tc BMS-181321 was performed in Langendorff buffer-perfused rat hearts using both bolus injections and constant infusion experiments (*4*). An inverse sigmoidal relationship was found between BMS retention and perfusate O_2 levels, suggesting a threshold level of tissue hypoxia required for tracer uptake. In an open-chest, extracorporally perfused swine model, ^{99m}Tc BMS-181321 retention was correlated with regional blood flow and regional metabolism during low flow ischemia (*5*). Washout of BMS was slower from the ischemic bed than from the

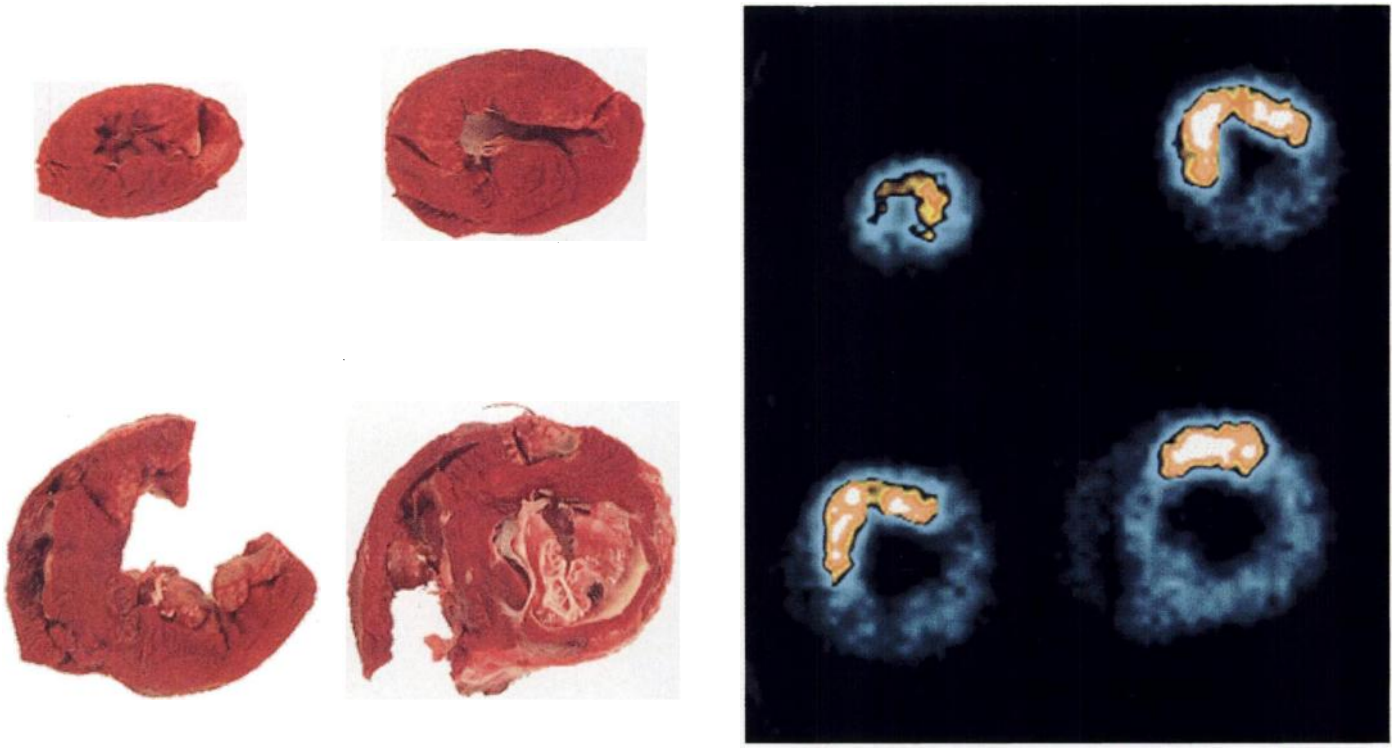

FIGURE 6. Myocardial slices from experiment shown in Figure 5. Triphenyl tetrazolium chloride- (TTC-) stained slices are on the left, and ex vivo-imaged slices are on the right. TTC-stained slices show small patches of TTC-negative staining confined to the endocardium of the risk region. A piece of tissue was removed from the bottom left slice. Imaged slices show tracer uptake in the anterior wall of the left ventricle (risk region).

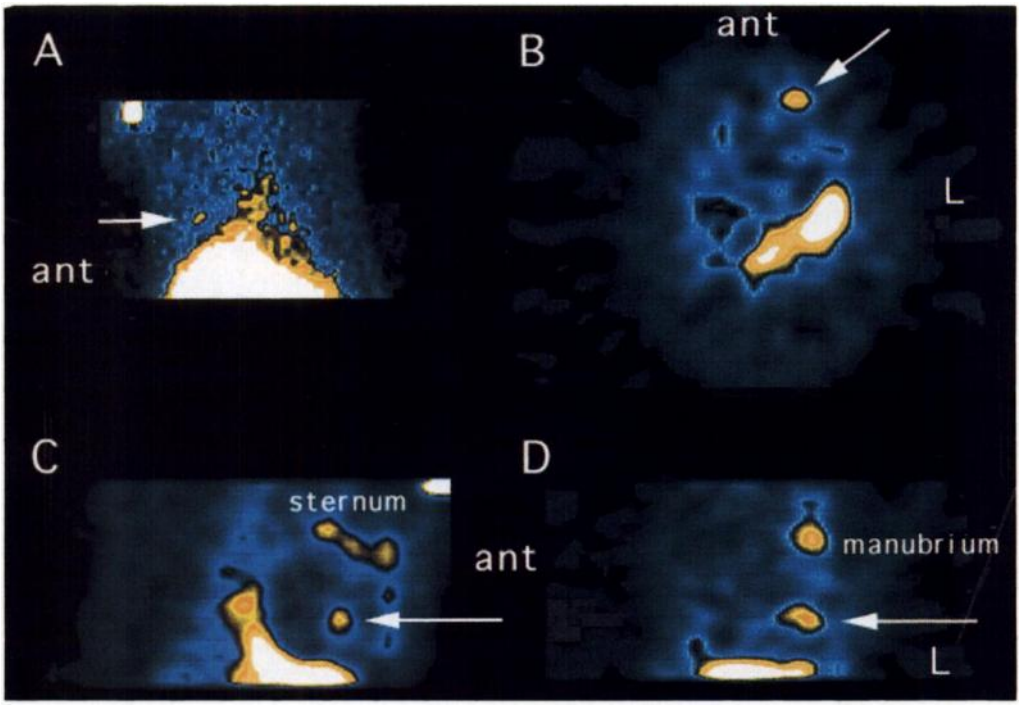

FIGURE 7. Left lateral projection image (A), transverse (B), sagittal (C) and coronal (D) tomographic reconstructed slices from an animal that sustained a small anterolateral infarction resulting from occlusion of a diagonal branch of the left anterior descending coronary artery by the catheter. The arrow points to BMS uptake seen on the left lateral projection image and on the tomographic slices. Focal tracer uptake is seen in the area corresponding to the anterior basal wall of the left ventricle (infarct region).

aerobically perfused bed. Planar scintigraphic images showed retention of tracer localized to the bed of the hypoperfused coronary artery. In this model, the tracer was delivered to the myocardium first and then recirculated via the right heart into the systemic circulation and therefore was not a model applicable to intravenous injection. However, other animal imaging experiments performed after intravenous tracer injection did not show sufficiently high tracer uptake in ischemic regions compared with nonischemic myocardial regions to show focal uptake on in vivo planar imaging (*8,9*). Positive in vivo images were achieved in an open-chest canine model of low flow ischemia with the improved contrast resolution of SPECT imaging (*10*). However, these experimental results suggested that BMS-181321 did not show sufficient target-to-background ratios for clinically useful in vivo diagnostic imaging. Consequently, the nitroimidazole compound was altered by moving the side chain from the 1–6 position of the chelate ring and substituting an oxygen atom for the —CH_2 group in the 5 position. Initial animal experiments using this further refined nitroimidazole compound, called BMS-194796, showed improved focal uptake and retention in a low flow ischemia canine model when compared with BMS-181321 in the same preparation (*11*).

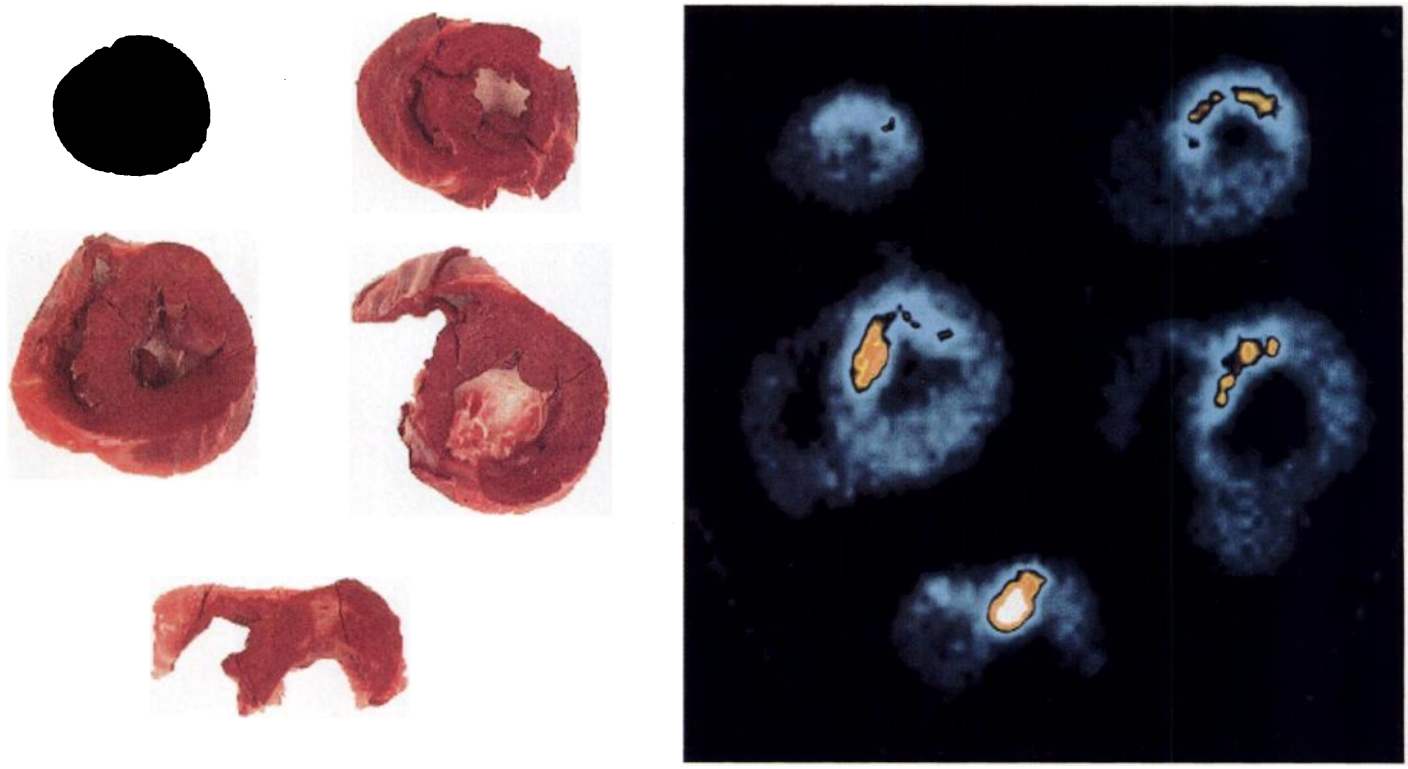

FIGURE 8. Myocardial slices from the experiment shown in Figure 7. Triphenyl tetrazolium chloride (TTC) stained slices are on left, and the ex vivo-imaged slices are on right. TTC-stained slices show TTC-positive staining throughout the risk region and a small area of TTC-negative staining on the basal slice. Imaged slices show focal tracer uptake on the same basal slices.

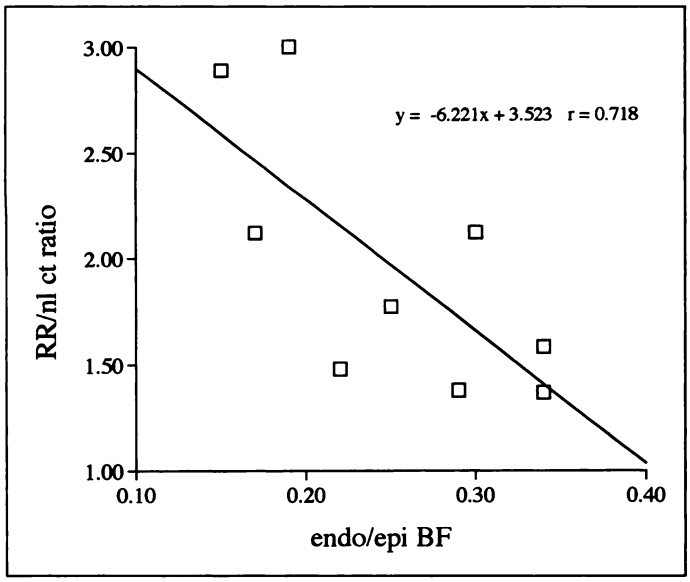

FIGURE 9. Correlation between risk region-to-normal count (RR/nl ct) ratio plotted against endo-to-epi myocardial blood flow (endo/epi BF) ratio in the risk region. The p value for the regression line was significant at $p < 0.05$ level.

Previous experiments evaluating the myocardial uptake and retention of radiolabeled nitroimidazole compounds have predominantly used either low flow ischemia or infarction models. This is the first study to report positive BMS images of BMS uptake and retention in myocardial ischemia after an intravenous injection of dose in a closed chest animal model that is close in size and coronary anatomy to humans. The domestic swine is a relevant animal model due to similarities in body surface area and coronary circulation with humans. The method of producing demand ischemia by pacing an animal with a plastic stenosis mounted on the tip of a PTCA catheter placed in the left anterior descending coronary artery was developed in the RIH cardiovascular laboratory (*12*). The lactate and flow data support the premise that during pacing, ischemia was achieved in 9 of the 12 experiments reported. In vivo imaging was positive in 8 of these 9 experiments, and the one false-negative experiment did not undergo SPECT imaging. BMS uptake was seen in the risk region on the slices imaged ex vivo in all 9 of these experiments. Of the 6 ischemic experiments that were TTC positive in the risk region, only 2 showed tracer uptake on in vivo imaging, and all experiments that showed some TTC-negative staining in the risk or infarct territories were positive. The extent of TTC-negative staining was minimal compared with the extent of BMS uptake. The presence of small scattered regions of subendocardial necrosis in the risk region indicates both that flows became very low in these regions during pacing and that these very low flows were associated with BMS retention.

These data support the premise that it is necessary to produce severe levels of ischemia to achieve sufficient concentrations of tracer to be visualized on in vivo imaging. In previous experiments using low flow ischemia achieved by reducing flow with cuff occluders and in which tissue levels of oxygen were measured, O_2 levels in the myocardium taking up BMS were in the range of 2–6 torr, in which normoxic myocardial O_2 levels were 20–25 torr (*17*). The results of this present experiment therefore appear to further support the premise that in addition to washout from normoxic myocardium, fairly low levels of tissue oxygen must be achieved to allow sufficient retention of tracer to be visualized on in vivo imaging.

The domestic swine has a large number of diagonal branches that come off the proximal left anterior descending coronary. Despite careful attempts to place the stenosis at a site free from a take-off of a diagnonal branch, at the completion of the experiment, the plastic stenosis was found to have moved into a position that has occluded a diagonal branch in 4 animals. Although it was the design of this study to produce ischemia alone, the findings of small infarcts in the distribution of branch vessel in some of these experiments do not detract from the overall value of the data to demonstrate positive in vivo images. Previous studies have shown BMS retention in infarct border zones, where myocytes are ischemic but viable (*8,18*). The branch vessel infarcts in this study were very small. Due to photon scatter and limited resolution of the gamma camera, it was impossible to separate border and central infarction in these small regions.

CONCLUSION

BMS-194796 has potential as a hot spot imaging agent for in vivo imaging of myocardial ischemia. Focal uptake of tracer was seen in either the risk region and/or distribution of a branch vessel infarction on either planar and/or SPECT imaging within 2–3 hr following tracer injection in all but one experiment. This tracer may have applications in acute ischemic syndromes.

ACKNOWLEDGMENTS

This work was supported by a grant from Bracco Diagnostics (Princeton, NJ).

REFERENCES

1. Chan YW, Linder KE, Jayatilik PG, et al. In-vitro studies on the hypoxic retention of a novel technetium-99m labeled nitroimidazole in rat heart [Abstract]. *J Nucl Med* 1994;35:18P.
2. Rauth AM. Pharmacology and toxicology of sensitizers: mechanism studies. *Int J Radiat Oncol Biol Phys* 1984;10:1293–1300.
3. Rumsey WL, Cyr JE, Nara RK. A novel ^{99m}Tc-labeled nitroheterocycle capable of identification of hypoxia in heart. *Biochem Biophys Res Commun* 1993;193:1239–1246.
4. Ng CK, Sinusas AJ, Zaret BL, Soufer R. Kinetic analysis of technetium-99m-labeled nitroimidazole (BMS-181321) as a tracer of myocardial hypoxia. *Circulation* 1995; 92:1261–1268.
5. Stone CK, Mulnix T, Nickles RJ, et al. Myocardial kinetics of a putative hypoxic tissue marker, ^{99m}Tc-labeled nitroimidazole (BMS-181321), after regional ischemia and reperfusion. *Circulation* 1995;92:1246–1253.
6. Okada RD, Nguyen KN, Strauss HW, Johnson G. Effects of low flow and hypoxia on myocardial retention of technetium-99m BMS181321. *Eur J Nucl Med* 1996;23:443–447.
7. Rumsey WL, Patel B, Linder KE. Effect of graded hypoxia on retention of technetium-99m-nitroheterocycle in perfused rat heart. *J Nucl Med* 1995;36:632–636.
8. Johnson LL, Tauxe EL, Straeter-Knowlen I, et al. Hypoxic infarct border zone delineated ex-vivo in a canine infarct model using a technetium-99m labeled nitroheterocycle [Abstract]. *J Nucl Med* 1995;36:137P.
9. Shi CQ, Sinusas AJ, Dione DP, et al. Technetium-99m-nitroimidazole (BMSS181321): a positive imaging agent for detecting myocardial ischemia. *J Nucl Med* 1995;36:1078–1086.
10. Rumsey WL, Kuczynski B, Patel B, et al. SPECT imaging of ischemic myocardium using a technetium-99m-nitroimidazole ligand. *J Nucl Med* 1995;36:1445–1450.
11. Kucynski B, Linder K, Patel B, et al. Dual isotope imaging of Tc-99m BMS-194796 and TI-201 in dog coronary artery1 stenosis model [Abstract]. *J Nucl Cardiol* 1995;S28.
12. Gewirtz H, Most AS. Production of a critical coronary arterial stenosis in closed chest laboratory animals. *Am J Cardiol* 1981;47:589–596.
13. Hale SL, Alker KF, Kloner RA. Evaluation of nonradioactive, colored microspheres for measurement of regional myocardial blood flow in dogs. *Circulation* 1988;78: 428–434.
14. Kobayashi N, Kobayashi K, Kouno K, Horinaka S, Shigeru Y. Effects of intra-atrial injection of colored microspheres on systemic hemodynamics and regional blood flow in rats. *Am J Physiol 1994:H1910–H1917.*
15. Vivaldi MT, Kloner RA, Schoen FJ. Triphenyltetrazolium staining of irreversible ischemic injury after coronary artery occlusion in rats. *Am J Pathol* 1985;121:522–530.
16. DiRocco RJ, Kuczynski BL, Pirro JP, et al. Imaging ischemic tissue at risk of infarction during stroke. *J Cereb Blood Flow Metab* 1993;13:755–762.
17. Nunn A, Linder K, Strauss HW. Nitroimidazoles and imaging hypoxia. *Eur J Nucl Med* 1995;22:265–280.
18. Di Rocco RJ, Bauer AA, Pirro JP, et al. Delineation of the border zone of ischemic rabbit myocardium by a technetium-labeled nitroimidazole. *Nucl Med Biol* 1997;24: 201–207.