Myocardial Uptake of a ^{99m}Tc-Nitroheterocycle in a Swine Model of Occlusion and Reperfusion

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The purpose of this study was to evaluate the window for scan positivity of the radiolabeled nitroheterocycle ^{99m}Tc–BRU-59-21 in the peri-ischemic period using a swine model of occlusion and reperfusion. **Methods:** A balloon catheter was placed in the left anterior descending coronary artery in each of 19 domestic swine. Blood flow and hemodynamic measurements were made at baseline, during occlusion, and at 15 and 180 min after reperfusion. A dose of approximately 925 MBq ^{99m}Tc–BRU-59-21 was injected before a brief (6 min) period of coronary occlusion at the following times: 15 min (n = 2), 5 min (n = 2), and 2.2 min (n = 5). In 5 experiments the dose was injected 15 min after reperfusion. Animals underwent SPECT imaging 3 h later. Animals were then killed, and hearts were removed, sliced, stained with triphenyl tetrazolium chloride, and imaged on the detector. **Results:** The risk region became ischemic during occlusion on the basis of severe reduction in blood flow and lactate production, but necrosis occurred in only 3 experiments. Focal tracer uptake was seen in the risk region in animals injected 5 and 2.2 min before occlusion but not in animals injected 15 min before occlusion and 15 min after reperfusion. **Conclusion:** The window for scan positivity for ^{99m}Tc–BRU-59-21 injected in the peri-ischemic period is short using this model of balloon occlusion and reperfusion in swine.

Key Words: nitroheterocycle; myocardium; ischemia; swine

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Nitroheterocycles are lipophilic compounds with an affinity for electrons. They diffuse readily across myocardial cell membranes and are retained in cells in the presence of low levels of O_2 (*1–9*). These compounds have been chelated to technetium for single-photon imaging. Slow tracer washout from normoxic tissue and high liver activity led to poor target-to-background ratios with some compounds (*10,11*). Changes in the chemical structures led to new tracers with improved imaging characteristics (*12,13*).

A potential clinical application for an imaging tracer characterized by avidity for hypoxia is use in patients with suspected acute coronary syndromes. To be clinically useful in patients coming to the emergency department with chest pain and with a high probability of an acute ischemic event, the imaging agent must have a fairly wide time window for scan positivity in the peri-ischemic period. The duration of reduced flow and oxygen debt may be short-lived. Patients frequently come to the emergency department after the symptoms of chest pain have resolved. However, episodes of ischemia may reoccur. The timing of these recurrent events cannot be predicted clinically and may be silent.

The pharmacokinetic properties of nitroheterocycles elucidated to date include diffuse myocardial uptake followed by washout from normoxic tissue and reduction with trapping (retention) in hypoxic tissue (*6–9*). Either prolonged low flow or demand ischemia has been used previously (*8,9*). In the clinical setting of acute ischemic syndromes, ischemia is associated with transient flow reduction. Under these conditions the relationship between the time of tracer injection and the onset of flow reduction may be crucial in determining scan positivity. Therefore, the objective of this study was to compare the time relationship between tracer injection and vessel occlusion with scan positivity in a swine model using the radiolabeled nitroimidazole compound ^{99m}Tc–BRU-59-21.

MATERIALS AND METHODS

Animal Preparation and Instrumentation

This study was performed within the guidelines of 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 domestic 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 thiopental. 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 L/min with room air and anesthetic gases. Arterial blood gases were obtained frequently to maintain constant pH, P_{O_2}, and P_{CO_2}. Heparin (225 IU/kg) was administered, and animals were heparinized as necessary by regular bolus-dose injections.

Surgical cutdowns were performed on the neck to expose the right internal carotid artery, the 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 pigtail catheter was advanced by fluoroscopic control from the right femoral artery into the left ventricle and in the retrograde direction across the mitral valve into the left atrium. This catheter was used to administer microspheres for measurement of regional

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myocardial blood flow and to measure left atrial pressures. An 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. An 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 the floppy end previously passed through the guiding catheter. A balloon catheter was advanced over a wire into the left anterior descending artery. Isoflurane and nitrous oxide were discontinued, and the animal was allowed to awaken sufficiently to breathe spontaneously and exhibit modest tremors. Intravenous sodium thiopental was then begun at 10–40 mL/h (120 mg/m) to maintain sedation and to ensure that the animal was free of pain.

Experimental Protocol

Arterial blood gases were monitored as needed to ensure appropriate ventilation. Initial measurement of the following variables was made: aortic and distal coronary pressure, left atrial pressure, and arterial and anterior interventricular vein sampling for lactate, O_2, and pH. The first set of colored microspheres (6 million) was injected (baseline). Colored microspheres (15 ± 0.43 μm) used for blood-flow determination are cross-linked polystyrene-divinylbenzene microspheres in 8 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 to strong acid and base solutions (*14,15*).

The balloon was occluded for 6 min. Occlusion was verified by a drop in distal coronary pressure. A second set of microspheres was injected during the fourth minute of occlusion. Two subsequent sets of microspheres were injected at 15 and 180 min after occlusion. ^{99m}Tc–BRU-59-21 was injected 15 min before occlusion (2 experiments), 5 min before occlusion (2 experiments), immediately before occlusion (130 s or approximately 2 circulation times) (5 experiments), or 15 min after reperfusion (5 experiments). Each animal was imaged 180 min after reperfusion. Blood-flow and hemodynamic measurements were made before occlusion, during occlusion, and at 15 and 180 min after reperfusion in all experiments.

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 (Whatman, Clifton, NJ) prespotted at the origin with acetonitrile and then immediately spotted with ^{99m}Tc–BRU-59-21. 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–BRU-59-21 was 962 ± 74 MBq.

Animals underwent planar and SPECT imaging using an Arc 3000 camera (ADAC Laboratories, Milpitas, CA) interfaced with a Nuclear Mac (NC Systems, Boulder, CO). Imaging was performed over a 180° orbit using a high-resolution collimator for 32 stops at 30 s/stop. Raw data were processed using a ramp and Weiner filters.

Killing and Pathology

The chest was opened and the coronary balloon was again inflated. 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 sodium thiopental, which was followed immediately by a lethal dose of KCl through the left atrial catheter. The proximal balloon location was marked with a tie. The heart was removed, washed, and sliced into 1-cm slices, which were imaged for 5 min on the camera detector and then immersed in 1.5% triphenyl tetrazolium chloride (TTC) at 37°C for 15 min (*16*). The heart slices were fixed by immersion 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.

Blood-Flow Measurements

Regional myocardial blood-flow values were measured using methods described by Hale et al. (*14*). Whole, weighted tissue samples of <3 g were hydolyzed in 2N NaOH solution overnight and then in tissue blood digest reagent II. Samples were centrifuged and the pellets were washed twice with microsphere counting reagent. The numbers of colored microspheres in the final tissue and reference blood preparations were counted manually using a hemocytometer counting slide. Either a total of 400 microspheres of each color was counted or counting was done until the sample was gone. Using colored microspheres, there is a mild overestimation of myocardial blood-flow measurements in the blood-flow ranges investigated in this study (*14*).

Tracer Uptake

Tissue uptake of radioactivity was determined both by calculating uptake as percentage 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 were drawn around the risk regions and normal regions. These regions were created using as guide a drawing of the TTC- and fluoroscein-stained heart slices, mounted in the identical orientation. To calculate percentage 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 the ratio of counts in the risk region to counts in the normal region.

Statistical Analysis

The within-group variation for the ischemic group was analyzed using a 1-way, repeated-measures ANOVA. To compare the ischemic and nonischemic groups, an independent sample *t* test was used.

RESULTS

Experiments Performed

Nineteen experiments were performed. Four animals died before completion of the protocol, and results from these experiments could not be used. Of the 15 remaining experiments, 2 animals were injected 15 min before occlusion, 3 were injected 5 min before occlusion, 5 were injected immediately (130 s or 2 tracer circulation times) before occlusion, and 5 were injected 15 min after occlusion.

Hemodynamic and Lactate Data

Hemodynamic and lactate data are summarized in Table 1. The heart rate did not change during the course of the

TABLE 1
Hemodynamic and Lactate Data

Variable	Before occlusion	Occlusion	15 min after occlusion	180 min after occlusion
Left atrial pressure (mm Hg)	2.9 ± 3.1	7.7 ± 3.3*	5.1 ± 5.5	4.3 ± 3.5
Heart rate (bpm)	97 ± 25	99 ± 18	94 ± 20	104 ± 30
Mean arterial pressure (mm Hg)	126 ± 16	110 ± 16*	122 ± 17	126 ± 22
AIV lactate extraction (%)	35 ± 16	−43 ± 49*	22 ± 16	30 ± 16

*$P < 0.01$ versus before occlusion.
bpm = beats/min; AIV = anterior interventricular vein.
Values are means ± SD for all experiments.

experimental protocol ($P = 0.27$). Mean arterial pressure fell during coronary occlusion ($P = 0.005$), and left atrial pressure rose during coronary occlusion ($P = 0.0003$). The percentage lactate extraction in the anterior interventricular vein went from 35% ± 16% before occlusion to −43% ± 49% during coronary occlusion to 22% ±16% and 30% ± 16% at 15 and 180 min after reperfusion, respectively ($P < 0.0001$).

Blood Flow

Blood-flow data are summarized in Table 2. Transmural blood flow in the normally perfused myocardium did not change significantly during the experimental protocol ($P = 0.87$). Transmural blood flow in the risk region fell significantly from 1.00 ± 0.27 mL/g/min before occlusion to 0.16 ± 0.16 mL/g/min during occlusion ($P < 0.0001$). Blood flow rose to 0.88 ± 0.33 mL/g/min at 15 min after reperfusion and to 0.92 ± 0.18 mL/g/min at 180 min after reperfusion. Similarly, endocardial blood flow did not fall significantly in the normally perfused myocardium during balloon occlusion ($P = 0.85$). Endocardial blood flow fell significantly in the risk region, going from 1.12 ± 0.28 mL/g/min before occlusion to 0.19 ± 0.25 mL/g/min during occlusion ($P < 0.001$) and returned to 1.00 ± 0.41 mL/g/min at 15 min after reperfusion and 1.01 ± 0.24 mL/g/min 180 min after reperfusion.

Pathology

Twelve of the 15 animals showed uniform TTC-positive staining in the risk region, and 3 animals showed TTC-negative staining in the risk region. One of the 3 animals injected with tracer 5 min before occlusion and 1 of the 5 animals injected immediately before occlusion showed small focal scattered areas of TTC-negative staining in the subendocardium of the risk region. One of the 5 animals injected 15 min after reperfusion showed a more confluent area of TTC-negative staining in the risk region, consistent with transmural infarction.

Imaging

In vivo SPECT images and ex vivo images of the myocardial slices from representative animals injected 15 and 5 min before occlusion, immediately before occlusion, and 15 min after reperfusion are displayed in Figures 1–4. Focal tracer uptake in the risk region was seen on reconstructed tomograms in all animals injected 5 min before occlusion and immediately before occlusion. None of the animals injected 15 min before occlusion showed focal tracer uptake in the risk region either on in vivo tomographic images or on ex vivo images of the heart slices. No region of focal tracer uptake was seen in 4 of the 5 animals injected 15 min after reperfusion. The 1 animal that showed focal tracer uptake in the risk region also had TTC-negative staining in the risk region, which indicated necrosis. For 15 min before occlusion, 5 min before occlusion, immediately before occlusion, and 15 min after occlusion, the average ratios were 1.45, 2.27, 4.45, and 1.13, respectively. Tracer uptake as the percentage injected dose for animals injected 5 min before occlusion was 4% and for those injected immediately before occlusion was 6%.

DISCUSSION

Radiotracers taken up in the distribution of myocardial blood flow or tissue viability (or both), such as ^{201}Tl and ^{99m}Tc-sestamibi, identify ischemic myocardium on the basis of reduced blood supply. However, the presence of a

TABLE 2
Blood-Flow Data

Variable	Before occlusion	Occlusion	15 min after occlusion	180 min after occlusion
Transmural BF (mL/g/min)				
Normal region	1.05 ± 0.27	1.00 ± 0.37	1.07 ± 0.29	1.10 ± 0.22
Risk region	1.00 ± 0.27	0.16 ± 0.16*	0.88 ± 0.33	0.92 ± 0.18
Endocardial BF (mL/g/min)				
Normal region	1.17 ± 0.33	1.04 ± 0.39	1.14 ± 0.34	1.18 ± 0.28
Risk region	1.12 ± 0.28	0.19 ± 0.25*	1.00 ± 0.41	1.01 ± 0.24

*$P < 0.0001$ versus before occlusion.
BF = myocardial blood flow.
Values are means ± SD for all experiments.

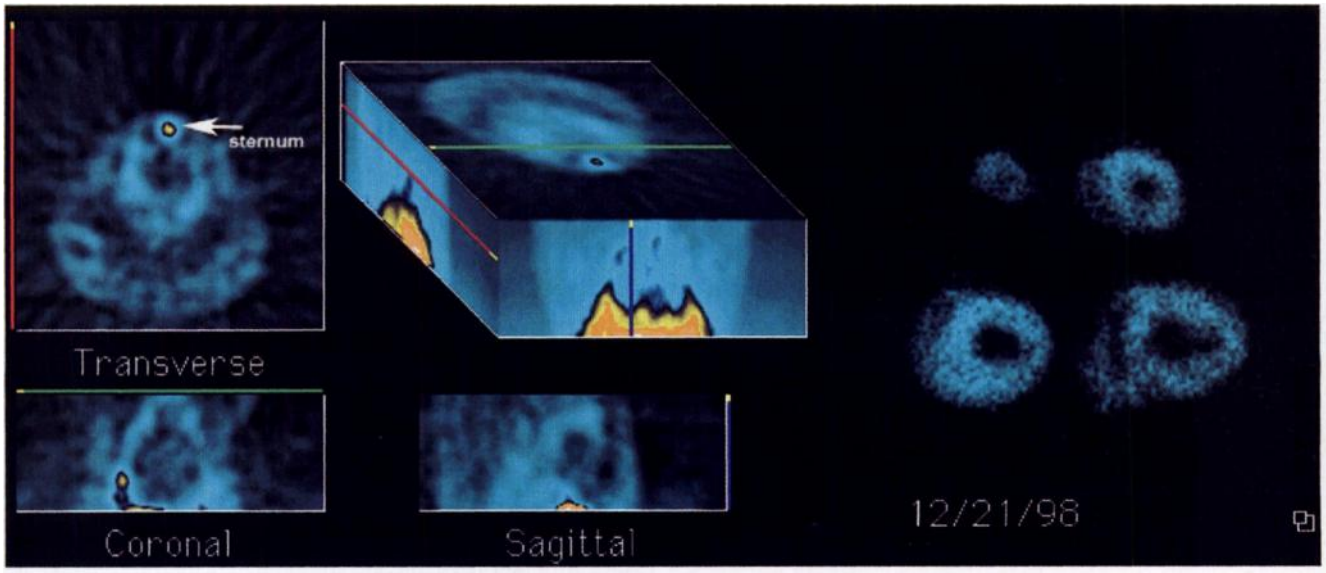

FIGURE 1. Images from animal injected 15 min before occlusion. Red, green, and blue lines on cube display correspond to plane chosen to display transverse, coronal, and sagittal slices. Ex vivo imaged heart slices are displayed on right. No focal tracer uptake is seen in region of heart.

perfusion defect is not specific for ischemia because it may also represent scar. There are several clinical situations in which it would be very useful to have a hot-spot radiotracer that is specific for myocardial hypoxia or ischemia. The nitroimidazoles represent a series of compounds with interesting properties that have led to their application as radiation sensitizers, antibiotics, and, more recently, radiolabeled hypoxia imaging agents (*1,2*).

The first such agent developed was the positron-emitting tracer ^{18}F-misonidazole (*17*). The development of ligands for technetium labeling to nitroimidazoles has led to several radiotracers that have undergone experimental study. One of the first ^{99m}Tc-nitroimidazole tracers developed was BMS-181321. Using an extracorporeal perfused swine model, Stone et al. (*7*) reported uptake and retention in ischemic myocardium. The mechanism for intracellular retention of nitroimidazoles is not fully understood. It is believed that nitroimidazole compounds undergo reduction with the formation of products that bind to intracellular elements and remain trapped in hypoxic tissues. The major limitation to BMS-181321 was delayed washout from normoxic tissue with resultant low count ratios in the ischemic-to-normal myocardial zones and failure to detect the ischemic zone on in vivo imaging. In the study reported by Shi et al. (*10*), the

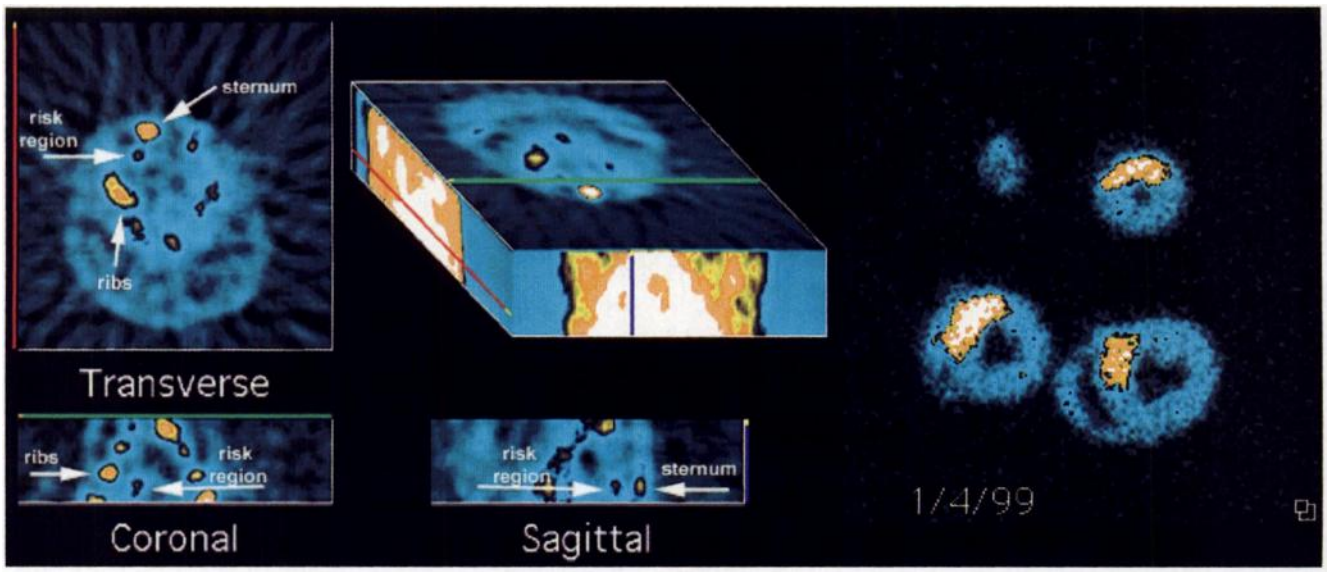

FIGURE 2. Images from animal injected 5 min before occlusion. Red, green, and blue lines on cube display correspond to plane chosen to display the transverse, coronal, and sagittal slices. Ex vivo imaged heart slices are displayed on right. White arrows point to focal uptake in risk region and correspond to anteroseptal, apical focal uptake seen on ex vivo slices. Note bone and liver uptake. Upper threshold was lowered to reveal this faint uptake. Rescaling accentuated bone uptake (arrows).

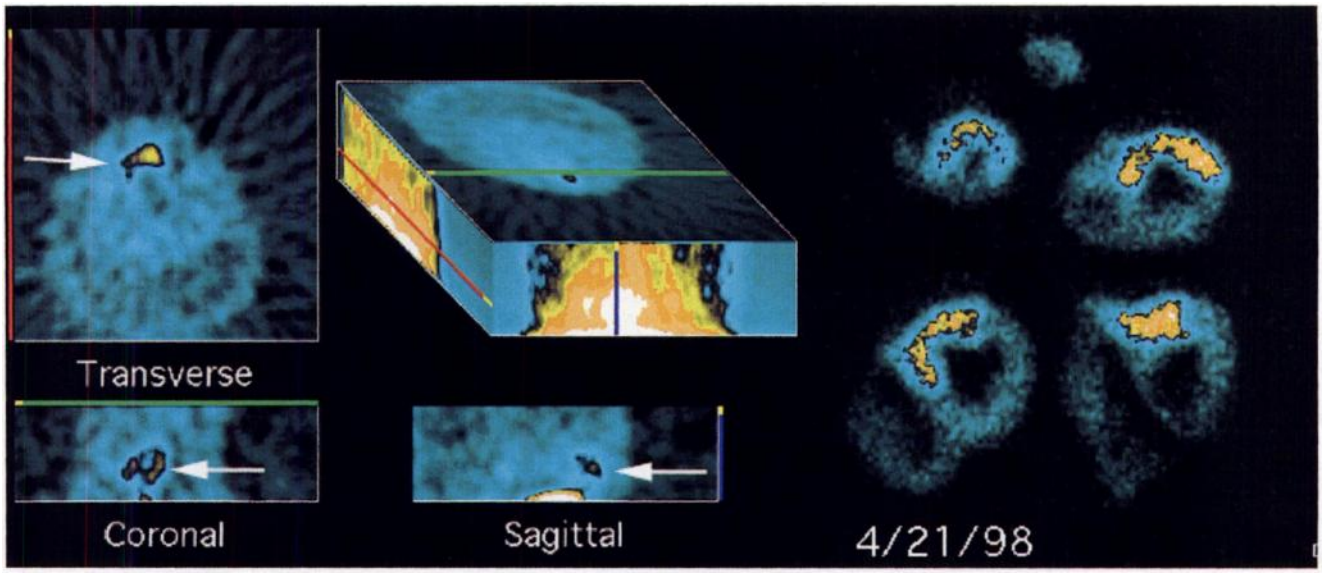

FIGURE 3. Images from animal injected 130 s before occlusion. Red, green, and blue lines on cube display correspond to plane chosen to display transverse, coronal, and sagittal slices. Ex vivo imaged heart slices are displayed on right. White arrows point to focal uptake in risk region and correspond to anteroseptal, apical focal uptake seen on ex vivo slices. Target-to-background ratio is higher in this animal than that in animal injected 5 min before occlusion (Fig. 2).

ischemic-to-normal count ratio was 1.7. The upscatter from hepatic activity also detracted from focal myocardial uptake visualization in this study.

To overcome these limitations several chemical modifications of the original compound were made, which has led to 2 other ^{99m}Tc-nitroimidazole compounds. One of the modifications was to remove the 2-nitroimidazole moiety from the parent nitroimidazole compound, resulting in ^{99}Tc-HL91. Okada et al. (*13*) reported the results of an experimental study using an open-chest canine model of prolonged low flow produced by a left circumflex occluder. They documented hot spots in the risk region within 60 min after tracer injection with continued improvement over 4 h. The ischemic-to-normal count ratio in their study was 3.0, higher than that reported for BMS-181321. Another approach to alter the parent nitroimidazole compound was to move the nitroimidazole moiety to the 6 position on the chelate ring and substitute an oxygen atom for the $-CH_2$ group in the 5 position (Fig. 5). This compound (BMS-194796 or BRU-59-21) has been shown to have imaging characteristics that are improved over those of BMS-181321 (*12*).

Two experimental imaging studies evaluating nitroimidazoles have used models of prolonged low flow (*11,13*), and 2 studies have used models of demand ischemia (*10,12*). Shi

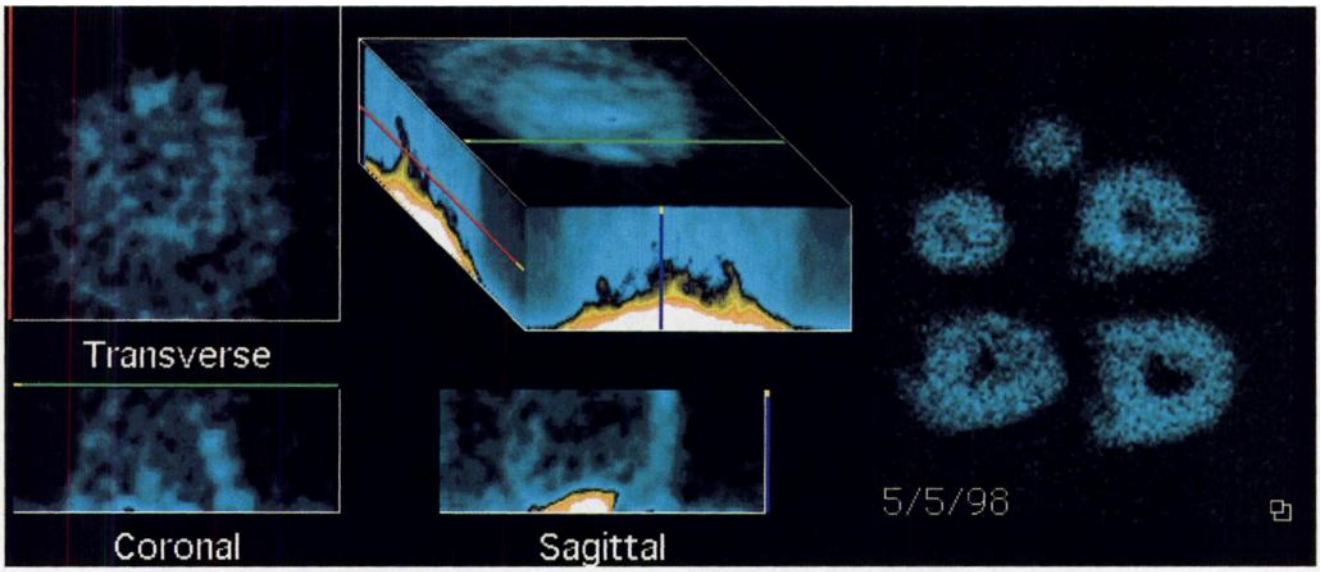

FIGURE 4. Images from animal injected 15 min after reperfusion. Red, green, and blue lines on cube display correspond to plane chosen to display transverse, coronal, and sagittal slices. Ex vivo imaged heart slices are displayed on right. No focal tracer uptake is seen in region of heart.

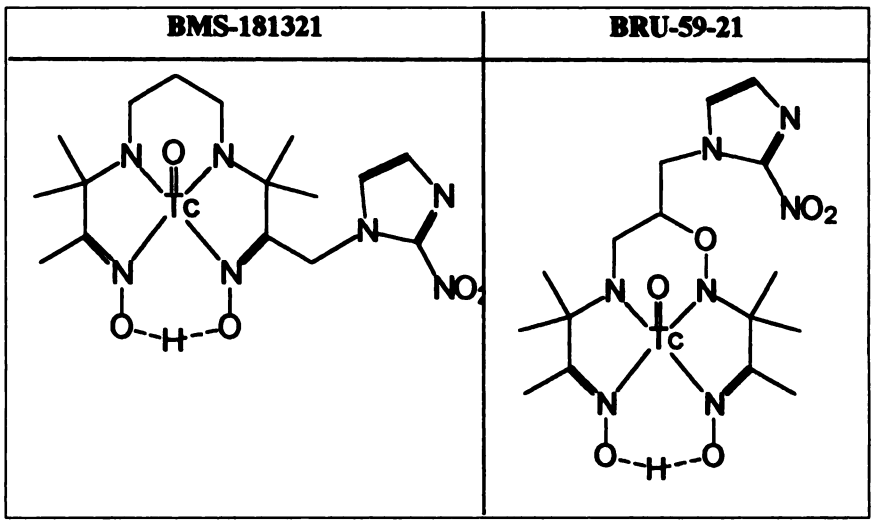

FIGURE 5. Chemical structures of nitroimidazole compounds BMS-181321 (left) and BRU-59-21 (right).

et al. (*10*), using an open-chest canine model of partial coronary occlusion plus pacing, documented focal uptake of BMS-181321 in the risk region on ex vivo imaging, but in vivo planar imaging was unsuccessful because of high liver activity. Focal uptake of BRU-59-21 in the risk region was documented on in vivo SPECT imaging in a closed-chest swine model in which demand ischemia was produced by pacing an animal with reduced resting flow caused by a plastic stenosis mounted on the tip of a catheter (*12*). Focal uptake was best seen when imaging was performed at least 3 h after tracer injection and in animals that showed evidence by TTC of scattered myocyte necrosis (*12*). This study was designed to further investigate this agent in an animal model of ischemia that is relevant to clinical situations.

In the clinical setting it is frequently not feasible to inject a radiotracer at the very moment of ischemia when the latter happens transiently or silently (or both), such as during unstable angina or acute coronary syndrome. Because of the recognized property of the nitroimidazole compounds to be taken up by normoxic tissue but retained by hypoxic tissue, we hypothesized that positive scans may be seen if the tracer is injected before the ischemic event. After the ischemic event, a tracer would be taken up only if there is "memory" for ischemia. The results of this study suggest that the window for scan positivity using ^{99m}Tc–BRU-59-21 is narrow around the ischemic episode. When animals were injected 5 min and immediately before vessel occlusion, in vivo SPECT scans showed focal uptake in the risk region in the absence of any TTC evidence of necrosis. However, when tracer was injected 15 min before occlusion or 15 min after reperfusion, all scans were negative, with the exception of 1 animal showing infarction. Ischemia during occlusion was documented by severe blood-flow reduction and net lactate production in the risk region in all scan-negative experiments. The risk region-to-normal count ratios for the ex vivo imaged slices for the animals injected 5 min before vessel occlusion were slightly lower than those reported by Okada (*13*) using ^{99}Tc-HL91. The count ratios for the ex vivo imaged slices for animals injected immediately before occlusion were higher.

Three of the animals showed necrosis in the risk region that resulted from the occlusion. Several experimental studies have shown uptake of nitroimidazoles in low-flow hypoxic regions in peri-infarction tissue (*18,19*). This represents the most likely explanation for the 1 positive scan seen in an animal injected 15 min after reperfusion. The other 2 animals showing necrosis and positive focal uptake were seen at 5 min and immediately before occlusion. In both cases, other animals injected at the same time were TTC negative and scan positive.

CONCLUSION

In a closed-chest swine model of occlusion and reperfusion, the ^{99m}Tc-nitroimidazole compound BRU-59-21 showed uptake and retention in the risk region when injected 5 min or immediately before coronary occlusion or reperfusion that were sufficient to be visualized on in vivo SPECT imaging. However, the nitroimidazole compound did not show focal uptake and retention when injected 15 min before occlusion or 15 min after reperfusion, despite evidence for ischemia during coronary occlusion. The window for scan positivity in the peri-ischemic period appears to be narrow. These data suggest that ^{99m}Tc–BRU-59-21 must be injected immediately before or at the time of reduced flow to detect myocardial ischemia accurately.

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