SPECT Imaging of Ischemic Myocardium Using a Technetium-99m-Nitroimidazole Ligand

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This investigation evaluates the efficacy of a ^{99m}Tc-labeled nitroimidazole (BMS-181321) in identifying oxygen-deprived tissue in two canine models of myocardial ischemia. **Methods:** For both models (A and B), epicardial microvascular oxygen pressure (mPO_2) was monitored by measuring the oxygen-dependent quenching of phosphorescence lifetime of Palladium meso-tetra (4-carboxyphenyl) porphine. In Model A (beagles, n = 5), BMS-181321 was administered intravenously and a distal branch of the left anterior descending coronary artery (LAD) was ligated completely 40 sec later. Ten minutes later, the ligature was released establishing tissue reoxygenation. In Model B, flow through the LAD was reduced until the mPO_2 was about 2 Torr. After bolus administration of BMS-181321 (50–60 mCi), coronary ischemia was continued for a residence period of up to 4 hr. **Results:** With Model A, SPECT reconstructions revealed a small ischemic area in three of five dogs, however, a transmural accumulation of the compound was evident in the autoradiograms from all dogs. In the two animals in which the defect was not observed by SPECT, the ischemic episode had nominal effects on the ratio of ±dp/dt (<4% change as compared to baseline values). In Model B, SPECT reconstructions showed positive images of the oxygen-deprived area within the mid- to apical regions of the left ventricle (n = 5). Autoradiographic analysis showed a transmural association with cells resulting in an ischemic-to-nonischemic ratio of 3.5 ± 0.4 (n = 4) for animals with similar residence times. **Conclusion:** The results from both models suggest that BMS-181321 provides a noninvasive marker of regional ischemia in the heart and that this compound may have clinical utility for detection of coronary artery disease.

Key Words: single-photon emission computed tomography; myocardial ischemia; technetium-99m-nitroimidazole

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It is generally accepted that cardiac muscle requires a continuous high level of oxygen delivery to maintain energy production via mitochondrial oxidative phosphorylation. A decrease in tissue oxygenation secondary to diminished coronary perfusion places the heart at serious risk for irreversible muscle damage and a loss of cardiac function. To restore adequate oxygen delivery, appropriate and timely therapeutic interventions partially depend on the clinician's ability to define the area of low oxygen. Previously, the ability to determine regional distribution of oxygen in the heart with noninvasive techniques has not been possible. Perfusion imaging, commonly used to detect coronary vessel disease, is at best an indirect measure of tissue oxygenation.

In view of the importance of evaluating myocardial oxygenation noninvasively, a new radiopharmaceutical, BMS-181321, that can localize in hypoxic cells has been developed. This electron affinic compound, which is a ^{99m}Tc-labeled nitroheterocycle, has been shown to associate preferentially with hypoxic cardiac myocytes in vitro (*1*) and with cerebral tissue rendered ischemic by occlusion of the middle cerebral artery (*2*). In the latter study, SPECT images provided a positive indication of ischemia unlike that normally obtained with perfusion imaging modalities. The purpose of this study was to evaluate the efficacy of BMS-181321 for detecting ischemic zones in the heart in vivo using a gamma camera. In two separate canine models of ischemia, SPECT reconstructions demarcated the areas of oxygen deprivation.

MATERIALS AND METHODS

Purebred beagle (11–14 kg) or mongrel dogs (15–18 kg) were anesthetized with sodium pentobarbital (50 mg/kg i.p., Nembutal, Abbott, Chicago, IL) and instrumented for measurements of aortic and left intraventricular pressures, dp/dt and cardiac electrical activity. A femoral vein was cannulated for administration of additional anesthesia (when needed), BMS-181321 (50–63 mCi in 1 ml) and Pd-meso-Tetra (4-carboxyphenyl) porphine (Porphyrin Products, Logan, Utah; 100 mg in saline containing 6% bovine serum albumin). For timed collection of arterial blood (100-μl aliquots), the other femoral artery was also cannulated. A tracheal cannula was inserted and the animal was ventilated at rates sufficient to produce normoxemia (arterial pO_2 = 85–95 Torr) and normal values of arterial pH (7.3–7.38, pCO_2 = 37–42 Torr). The heart was exposed by thoracotomy between the fourth and fifth ribs. The pericardium was used to cradle the heart to minimize motion imparted by ventilation.

Measurement of microvascular oxygen pressures (mPO_2) was made using a method based on phosphorescence quenching by molecular oxygen. This method of determining oxygen in vitro and in vivo has been previously described (*3–6*). In this study, a fiber-optic bundle capable of transmitting light to and from the

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tissue was placed about 2–3 mm from the surface of the heart for determination of epicardial tissue supplied by the left anterior descending artery (LAD). The position of the fiber-optic bundle was stabilized using aluminum bars connected to the surgical table. The optical cable was coupled to both a xenon flashlamp assembly and a photomultiplier tube for excitation of the lumiphor (>510 nm) and detection of the emitted light (>650 nm), respectively. The exciting light was directed towards an area free of surface conduit vessels. This system (Oxyspot, Medical Systems Inc., Greenvale, NY) was interfaced with a computer for online monitoring of phosphorescence lifetimes and oxygen pressures.

Protocol 1: Transient Ischemia

Two experimental protocols were used. For the first protocol, referred to as transient ischemia, a small branch of the LAD in beagles was isolated distal to its major conduit segment. A suture (ribbon floss) was placed around this small vessel and both ends of the suture were fed through a piece of polyethelene tubing. By lifting the suture away from the heart and depressing the tubing towards the heart, complete cessation of flow resulted, indicated by the rapid fall in mPO_2 to near zero. This protocol proceeded as follows: BMS-181321 was administered intravenously as a bolus and 40 sec later, the branch of the LAD was occluded. After a brief period (maximum duration was 10 min), the ligature was released, establishing reoxygenation of the affected area. A previous study (*1*) indicated that a period of 10 min was necessary for marked association of the compound to occur with isolated hypoxic cardiac myocytes.

In preliminary studies using the gamma camera, bolus injection of BMS-181321 into a peripherial vein resulted in optimal resolution of the normal heart at about 40 sec postinjection. This duration was therefore chosen as the period of time to be used prior to complete occlusion of the LAD as previously described. Additional preliminary studies showed that administration of the compound following cessation of coronary flow markedly impeded delivery of the agent to the affected myocardium due in large part to the lack of sufficient arterial input.

Protocol 2: Chronic Ischemia

For the second protocol, referred to as chronic ischemia, a plastic screw-type occluder was positioned around the LAD near its origin. Ischemia was induced by sufficient tightening of the screw to reduce coronary flow until microvasculature PO_2 was decreased to about 2 Torr. The animals (mongrel dogs) were then maintained at this level of mPO_2 for the remainder of the experiment. In these cases, the animal was stabilized at this low level of mPO_2 for about 8–10 min and then BMS-181321 was injected. In both studies, the radionuclide distribution within the animals was monitored with the gamma camera for a minimum of 1 hr postinjection or for a maximum of 4 hr.

For each protocol, SPECT images were acquired at 30 and 60 min postinjection and then at 1-hr intervals, providing a minimum of two but as many as five sets of images. An Elscint Helix gamma camera (Hackensack, NJ) with a high-resolution, parallel-hole collimator (APC-46) was used to accumulate frames at 10-sec intervals in continuous mode (rotation range and rotation step were 360° and 4°, respectively). Planar images were obtained immediately prior to SPECT acquisitions. The former were obtained using three different views: RAO-30, LAO-60 and anterior with frame time set at 10–60 sec.

After the animal was killed, the heart was dissected from the great vessels, removed from the thorax and rinsed in ice-cold saline. The heart was placed on the camera head and monitored for radioactivity. When imaging was complete, the heart was sliced into three sections corresponding to the apex, midventricle and base. These pieces were frozen, and cut into 40-μm sections using a cryostat thermostated at −15°C. The frozen sections were mounted on glass slides and were then exposed for 24 hr to x-ray film. Determination of differences in optical density due to retention of the nitroheterocycle were made with commercially available software (M1, Imaging Research, Inc., Toronto, Canada).

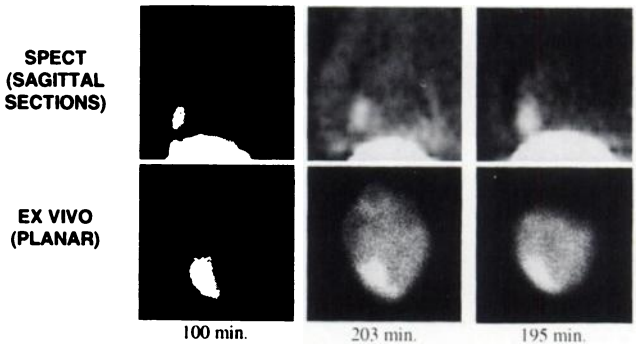

FIGURE 1. SPECT and ex vivo images of BMS-181321 distribution in the transient ischemic canine heart. SPECT images (sagittal plane displayed) were obtained at the times indicated postinjection at the base of each column. In the ex vivo images, the orientation of the heart places the LAD to the far left side.

Synthesis of BMS-181321 (oxo [[3,3,9,9-tetramethyl-1-(2-nitro-1H-imidazol-1-yl)-4,8-diazaundecane-2, 10-dione dioximato] (3-)-N, N′, N″, N‴]technetium) has been described previously (7). In brief, BMS 181321 was prepared by dissolving 2.0 mg PnAO-1,2-nitroimidazole ligand in 1.5 ml saline, 0.5 ml 0.1N $NaHCO_3$ and 0.5 ml of generator eluate (up to 100 mCi $^{99m}TcO_{-4}$). The reaction was initiated by addition of either 50 μl of a deoxygenated saturated stannous tartrate solution or with 100 μl of Techneplex (in 4 ml saline, final $[SnCl_2]$= 28 μM, Squibb Diagnostics, Princeton, NJ). The reaction was completed within 10 min at room temperature and the compound was used immediately after preparation. The radiochemical purity of BMS-181321 was always greater than 90% as detailed by Linder et al. (7).

RESULTS

Transient Ischemia Studies

Figure 1 (upper panels) shows examples of SPECT reconstructions obtained in the sagittal plane from three of five dogs given BMS-181321 just prior to occluding a small branch of the LAD for 10 min. Image acquisition was initiated between 100 and 203 min postinjection. The radioactivity was retained in the center lower left quandrant of each image, which corresponds to the left ventricle of the heart. In each dog, the level of radioactivity was markedly greater than that of the surrounding tissues with the exception of the liver located at the base of the images. The level of radioactivity in the heart was low relative to that in the liver. Nevertheless, the ischemic territory in the heart was readily apparent even though the tissue was reoxygenated to levels found prior to the ischemic insult. When the hearts were excised and placed atop the head of the gamma camera for planar imaging, radioactivity was emitted strongly from the midventricle to apex regions of the left

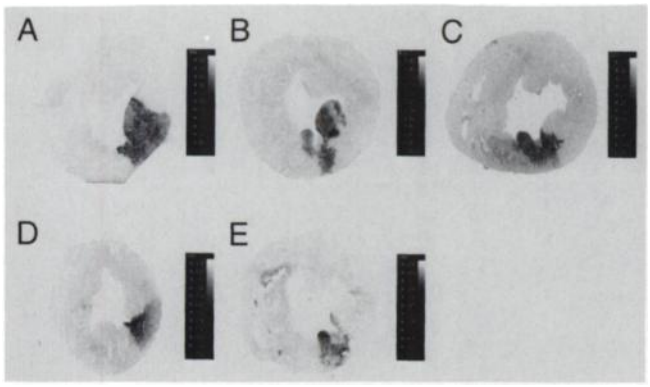

FIGURE 2. Autoradiograms of frozen sections obtained from canine ischemic hearts. The images in the upper panel correlate to the animals in Figure 1. The images in the lower panel were obtained from two animals from which SPECT images of the lesion were not possible.

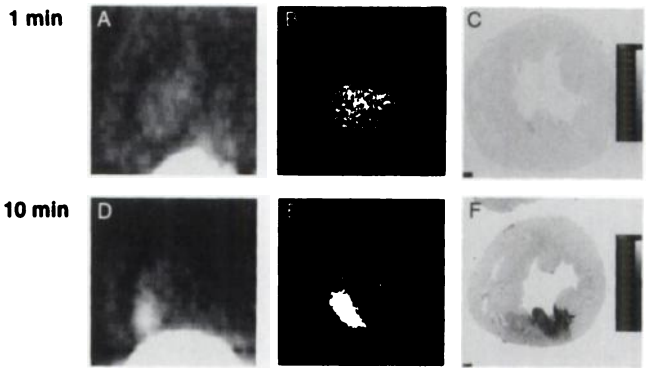

FIGURE 3. Comparison of the effects of 1- or 10-min ischemia on the distribution of BMS-181321 in canine hearts. Animals were subjected to either 1 (A–C) or 10 (D–F) min of total occlusion of the LAD. Images were obtained from animals at similar residence times, about 200 min postinjection. Images A and D represent SPECT reconstructions (sagittal view), whereas B and E refer to ex vivo images of hearts from the same animals. Images C and F are the respective autoradiograms.

ventricle. From these images, the lesion size was estimated to be small, from 6% to 29% of the total mass of each heart.

Autoradiography of frozen sections showed that the 10-min duration of ischemia resulted in transmural uptake of the nitroheterocycle. Optical density (i.e., radioactivity) was greater in endocardial muscle than in epicardial tissue (Fig. 2). The extent of nitroheterocycle retention represented a small area within the section of the left ventricle. The images in the upper panel (A–C) were obtained from the hearts in Figure 1, whereas those in the lower panel (D and E) were from dogs in which SPECT reconstructions or planar imaging did not provide evidence of an ischemic lesion. In the latter cases, the effect of LAD occlusion in these two hearts was not apparent from changes in the contractile efforts of the heart. In these two cases, the ratio of +dp/dt to −dp/dt was not altered markedly during ischemia as compared to values obtained prior to onset of the ischemic episode, i.e., 0.93 ± 0.17 (+dp/dt = 3125 ± 125 mmHg/sec; −dp/dt = 3500 ± 750 mmHg/sec) versus 0.83 ± 0.07 (+dp/dt = 3469 ± 94 mmHg/sec; −dp/dt = 3500 ± 750 mmHg/sec), respectively. By contrast, this ratio was markedly increased during ischemia, i.e., from 0.93 ± 0.01 (+dp/dt = 3038 ± 131; −dp/dt = 3390 ± 133 mmHg/sec) to 1.3 ± 0.05 (+dp/dt = 2583 ± 601; −dp/dt = 2000 ± 473 mmHg/sec) in those animals, providing evidence of an ischemic lesion after SPECT reconstructions.

Figure 3 compares the cardiac distribution of BMS-181321 in hearts subjected to either 1 or 10 min of transient ischemia. A 1-min occlusion did not result in differential uptake of the nitroheterocycle (Fig. 3A–C). In the example shown, which was typical of five experiments, the distribution of BMS-181321 is homogenous within the SPECT reconstruction, the ex vivo planar image and the respective autoradiogram. The absence of differentiation of the ischemic and nonischemic area in these hearts was not due to insufficient deoxygenation of the myocardial tissue. Figure 4 shows an example of the changes in microvascular oxygen pressure in the epicardium during the 1-min ischemic period. In this typical example, mPO_2 decreased from about 20 Torr during baseline conditions to nearly zero upon ligation of the LAD. After release of the ligature, tissue reperfusion was evident from the rise in mPO_2 to preischemic levels.

When the duration of ischemia was increased from 1 to 2 min, differentiation of the ischemic area was not evident. Three minutes of ischemia (n = 1) was sufficient time to provide marked localization of the compound as determined by autoradiographic analysis (ischemic-to-nonischemic area ratio was 1.91 after a residence time of 125 min). The lesion, however, was not defined by SPECT images (data not shown).

Chronic Ischemia Studies

Chronic ischemia was simulated by occluding the LAD to a level that resulted in low oxygen pressures in the epicardial microvasculature. For example, Figure 5 shows that mPO_2 decreased from about 20 Torr during baseline conditions to about 3 Torr after onset of ischemia. The animals were maintained at these low levels of oxygen for

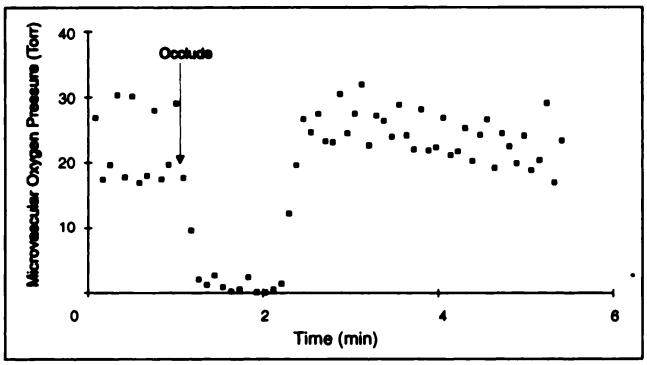

FIGURE 4. Changes in epicardial microvascular oxygen pressure resulting from transient ischemia. Microvascular oxygen pressures were monitored during a 1-min occlusion of a small branch of the LAD. The variation of the data is due to motion induced by the heart and lungs.

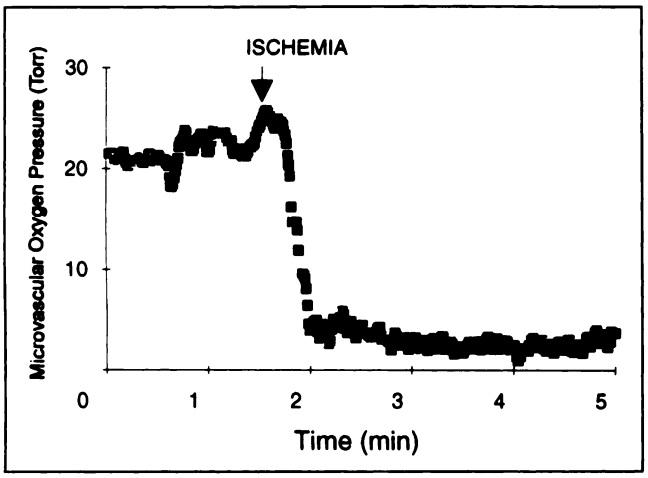

FIGURE 5. Changes in epicardial microvascular oxygen pressure resulting from chronic ischemia. Microvascular oxygen pressures were determined during the partial occlusion of the LAD near its origin. The squares represent the calculated values of oxygen pressure smoothed by averaging every 10 values.

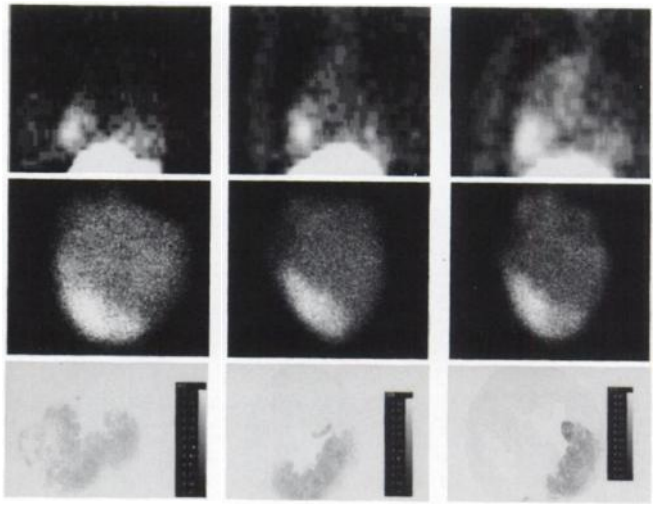

FIGURE 6. Images of BMS-181321 distribution in canine hearts subjected to chronic ischemia. SPECT reconstructions (upper panel), ex vivo images of intact heart (middle panels) and autoradiograms (lower panels) obtained from dogs subjected to partial occlusion of the LAD in three independent experiments. SPECT images (A–C, sagittal plane) were obtained at 160, 182 and 141 min postinjection, respectively.

the remainder of the experiment. In general, mPO_2 was 29.2 ± 4.4 Torr during baseline conditions and decreased to 2.4 ± 0.2 Torr (n = 5) during ischemia. The loss of tissue oxygen delivery resulted in a progressive diminution of contractililty, expressed as the ratio of +dp/dt to −dp/dt. Prior to ischemia, the ratio of +dp/dt to −dp/dt was 1.25 ± 0.11 and after LAD occlusion it quickly deteriorated to 1.09 ± 0.13 and continued to decline until at the conclusion of the experiments, it was 0.86 ± 0.06 (n = 5). This loss of cardiac function was most apparent in the rate of pressure development. Positive dp/dt decreased from 4750 ± 304 mmHg/sec obtained before occlusion to 3200 ± 222 mm Hg/sec at the end of the experiment, whereas negative dp/dt decreased from 3883 ± 255 to 3750 ± 220 mmHg/sec, respectively.

Administration of BMS-181321 resulted in a positive image of the ischemic lesion in all animals (n = 5). Typical examples of SPECT reconstructions (Fig. 6, sagittal view provided in upper panels) from three of five dogs show that radioactivity accumulated within the heart (i.e., <0.5% ID) similarly to that described above (lower left quadrant of images). Images obtained ex vivo (middle and lower panels) confirmed differential uptake of BMS-181321 within the heart, specifically in the region distal to the occluder. The autoradiograms show that radioactivity was prominently distributed in the endocardial and mesocardial areas of the left ventricle, involving the septum and in some cases, a small portion of the right ventricle. Autoradiographic analysis indicated that the increase in relative optical density within the ischemic area as compared to that within the normoxic territory was 3.5-fold ± 0.4 (n = 4) for animals killed at similar times.

Identification of the ischemic territory was readily apparent between 1 and 2 hr postinjection. Figure 7 shows sequential images of SPECT reconstructions obtained from the same animal and BMS-181321 clearance from both normal and ischemic tissue. Clearance from the bloodstream was rapid, displaying biexponential characteristics (data not shown). The half-time of the first phase was 1.2 ± 0.2 min, whereas that of the second phase was 79 ± 12 min (n = 5). These images show that the liver avidly accumulated BMS-181321. After initial rapid uptake, clearance from this tissue was slow and steady with a $t_{1/2}$ of about 172 ± 7 min.

DISCUSSION

The major finding of this study is that SPECT images of myocardial tissue deprived of normal oxygen delivery can be obtained after administration of BMS-181321. Although images of oxygen pressure distribution have been obtained from newborn piglets using an optical method based on the quenching of phosphoresence by oxygen (*8*), this method requires direct exposure of the heart to the camera. On the other hand, by directly measuring microvascular oxygen pressures in the LAD region of the heart, the impact of coronary flow reduction on tissue oxygenation and its relation to nitroimidazole retention could be evaluated. Two

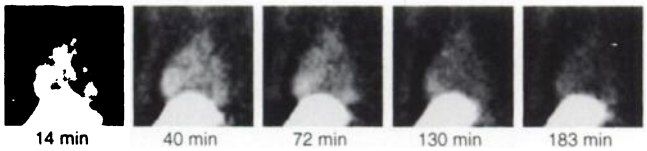

FIGURE 7. Sequential SPECT images of BMS-181321 uptake and clearance in ischemic canine heart. Images (sagittal plane) from the same dog were obtained at the times indicated at the base of each image after administration of BMS-181321.

independent models of ischemia, transient and chronic, were used in this study and provide evidence that a technetium-labeled 2-nitroimidazole can be used to image coronary ischemia.

The method of phosphorescence quenching to measure oxygen pressures in extracardiac tissues has been well documented by Wilson et al. (*3–6,8,9*). In blood, the phosphorescence of the administered lumiphor, in this case Pd-meso-Tetra (4-carboxyphenyl) porphine, is quenched by oxygen which is free in solution (not that bound to hemoglobin) as described by the Stern-Volmer relationship. The Stern-Volmer relation is described by the equation, $I^o/I = \tau^o/\tau = 1 + k_Q\tau^o[O_2]$; where I^o and τ^o are the phosphorescence intensity and lifetime, respectively, in the absence of oxygen; and I and τ are the phosphorescence intensity and lifetime at a given oxygen pressure. Lifetime values, not intensity levels, are used for the calculation of oxygen pressure. The oxygen probe is bound to albumin which serves to prevent self-quenching of the molecule and prevents the complex from escaping the vascular space (*4*). The oxygen measurements were weighted toward detection of longer phosphorescence lifetimes and therefore the measured values were primarily those obtained from blood within the capillaries and venules. The capillaries and venules not only have the lowest oxygen pressures in the vascular system (and therefore the longest phosphorescence lifetimes) but also contain a major fraction of the total blood volume. Since the phosphorescence lifetimes are independent of the probe concentration, changes in vascular volume or flow do not affect the calculated oxygen pressures. For measurements made on cardiac tissue in vivo, motion induced by contractile cycling and that from ventilatory efforts results in some variance of the oxygen values. Although it is now possible to partially correct for this motion by synchronizing the flashlamp emission with the cardiac cycle, we chose to simply dampen this noise by smoothing the data. The values of oxygen pressure obtained during baseline conditions were about 29 Torr, consistent with data reported for coronary sinus PO_2 (i.e., 15–20 Torr) by other investigators using alternate methods (*10*).

This study showed that BMS-181321 was preferentially retained in the heart in a model of transient ischemia. The duration of this occlusion was long (i.e., 10 min) and it was not possible to identify an ischemic lesion when the LAD was occluded for only 1 min. In a previous study, we provided evidence that the rise in association of BMS-181321 with anoxic cardiac myocytes in vitro occurs in a hyperbolic manner with nominal differentiation between normoxic and oxygen-deficient cells after 1 min of incubation (*1*). After 10 min of incubation, there was a marked accumulation of the nitroheterocycle and after 30 min, association of BMS-181321 with cells was near maximum levels. It is possible, therefore, that a period of ischemia in vivo of less than 10 min but greater than 1 min would be sufficient for marked retention of the nitroheterocycle within the ischemic lesion and for its identification by external imaging with the gamma camera.

The reason for the lack of differential uptake of BMS-181321 in tissue after only 1 min of ischemia in both in vivo and in vitro paradigms is not clear. One potential explanation is that access to the intracellular compartment is slow and limited by diffusion through the plasmalemmal membrane. Although association with cells seems to be rapid (*1*), this association may simply be due to residence of the nitroheterocycle on the outer aspect of the plasma membrane or within its lipid bilayer. Thus, the number of molecules available to the reactions of electron transfer may be too low during brief, 1 min or less, ischemic episodes. Alternatively, it is conceiveable that the amount of reduced products is too low for differentiation of ischemic and nonischemic tissue. There is evidence to suggest that these products bind covalently to macromolecules, impeding escape of the nitroheterocycle to the extracellular space (*11–15*). The present study suggests that radioactivity was cleared from the ischemic region of hearts, however, the precise nature of the radioactive species is not known. Fractionation of cardiac tissue following exposure to BMS-181321 indicated that a major portion of the radioactivity emitted from the cytosol (*16*). Moreover, the compound obtained from the cytosolic fraction appeared to have undergone conversion to a species more hydrophilic than that administered to the animal.

It is noteworthy that the amount of myocardial tissue affected by ischemia in the two animal models was markedly different. Although the ischemic territory was not quantitated using an exogenously administered marker, only a small distal branch of the LAD was occluded in the transient model and resulted in a physiological response different from that of the chronic model in which the LAD was occluded near its origin. Qualitative differences in size of the ischemic areas between the two models were apparent from the distribution of BMS-181321 in the hearts as shown in the autoradiograms. Despite the limited size of the ischemic zone produced in the transient model and the marked association of radioactivity in the liver, visualization of the ischemic lesion was possible after SPECT reconstructions of the images in three of the five subjects. In two animals, the apparent size of these lesions was too small for identification by external imaging. It may be possible, however, to identify lesions this small using a gamma camera with greater resolving power than that used in the present study.

Other researchers have obtained images of canine hearts with areas of low coronary flow using PET after injection of ^{18}F-fluoromisonidazole (*17*). In the latter case, it was necessary to allow considerable time to elapse—about 4 hr postinjection—in order to obtain good resolution of the ischemic territory. The authors suggested that this long postinjection delay was required due in part to the 4.5-hr plasma clearance half-time and the low levels of absolute binding of ^{18}F-flouromisonidazole to hypoxic tissue. By contrast, using BMS-181321 as the marker, images of the

ischemic area were possible in the present study at about 1 hr postinjection and these images were well resolved at 2 hr postinjection. Although both nitroheterocycles are 2-nitroimidazoles and both misonidazole and BMS-181321 display similar reduction characteristics (*7*), association of fluoromisonidazole with cardiac myocytes in vitro was less than that of BMS-181321 (*1*). The latter phenomenon is due, at least in part, to the greater lipophilicity of BMS-181321 as compared to that of fluoromisonidazole (*18*). Clearance of BMS-181321 from the circulation is rapid—$t_{1/2}$ of the first phase is about 1 min—promoting efflux of the compound from tissue. This latter factor might also contribute to the improved kinetics of imaging found with BMS-181321.

In previous studies, it has been shown that BMS-181321 and ^{18}F-fluoromisonidazole were not retained in infarcted areas of the brain (*2*) or heart (*17*), whereas marked localization was evident in ischemic but viable tissues. Postmortem analysis of viability of the ischemic tissue was not performed in the present investigation. It should be noted, however, that oxygenation of the affected epicardium was re-established to pre-insult levels in animals subjected to transient ischemia. These results suggest that, in general, these cells were metabolically viable. Treatment of cardiac myocytes with either uncouplers of oxidative phosphorylation or with cyanide in the presence of oxygen does not result in preferential uptake of BMS-181321 (*1*). Both of these treatments are known to severely compromise cell viability. It cannot be ruled out, however, that some cardiac myocytes—especially those within the endocardial regions—of animals from the present study may have undergone necrosis as a result of oxygen deprivation and that uptake of the nitroheterocycle occurred in these cells.

CONCLUSION

BMS-181321 has been found to be useful for the demarcation of areas within the canine heart that were low in oxygen pressure. Corroboration of the low oxygen pressure zones was made directly using phosphorescence quenching. External imaging of the ischemic lesions using SPECT was possible either early or later after injection of the isotopic compound, although clearance of the compound from the ischemic territory was evident from serial acquisition of images. Unlike conventional markers of coronary perfusion (i.e., thallium), BMS-181321 provides a positive image of oxygen deprivation in the heart. These studies suggest that BMS-181321 may be a clinically useful marker of ischemia resulting from coronary artery disease.

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