Kinetics of a Putative Hypoxic Tissue Marker, Technetium-99m-Nitroimidazole (BMS181321), in Normoxic, Hypoxic, Ischemic and Stunned Myocardium

Hideo Kusuoka, Katsuji Hashimoto, Kazuki Fukuchi and Tsunehiko Nishimura

Division of Tracer Kinetics, Biomedical Research Center, Osaka University Medical School, Suita Osaka 565 Japan

This study focused on the kinetics of the newly developed ^{99m}Tc-nitroimidazole, propyleneamine oxime-1,2-nitroimidazole (BMS181321) in the different setting of myocardial perfusion states and oxygenation levels, and compared the kinetics of BMS181321 with those of other technetium analogues. **Methods:** The kinetics of BMS181321 were evaluated in isolated perfused rat hearts. Technetium-99m-hexamethyl propyleneamine oxime (HMPAO) and a non-nitroimidazole-containing analogue of BMS181321 (6-methyl propyleneamine oxime; PAO-6-Me) were used to compare their kinetics with those of BMS181321. **Results:** BMS181321 cleared quickly from normoxic hearts and the retention in the myocardium 10 min after injection was 0.84% ± 0.04% ID/g wet wt (mean ± s.e.m.). In contrast, BMS181321 was retained after reperfusion when it was injected before ischemia; the uptake in the myocardium 10 min after reperfusion was significantly greater than in controls (23.9% ± 3.9% ID/g wt, $p < 0.05$). **Conclusions:** These results indicate that ^{99m}Tc-BMS181321 is well trapped in ischemic myocardium and moderately trapped in hypoxic myocardium, but washed out quickly in stunned myocardium. The residence time influences the amount retained.

Key Words: nitroimidazole; technetium-99m; myocardium; hypoxia; stunning

J Nucl Med 1994; 35:1371–1376

The nitroimidazoles have been studied as radiosensitizers of hypoxic regions in tumors (*1*). Recently, it has been shown that fluorinated or iodinated analogues of 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (misonidazole) accumulate in ischemic myocardium at a rate related inversely to tissue oxygen content (*2–5*). These results support the idea that nitroimidazoles may potentially be used to identify hypoxic myocardium.

A new ^{99m}Tc analogue of misonidazole, propyleneamine oxime-1,2-nitroimidazole (BMS181321, Fig. 1A) has been developed to detect hypoxic areas in the myocardium (*6,7*). The present study focused on characterizing the kinetics of BMS181321 in isolated, perfused rat hearts during normoxia, hypoxia or ischemia. The kinetics in myocardium reperfused after a brief period of ischemia (stunned myocardium, (*8*)) were also investigated. Technetium-99m-hexamethyl propyleneamine oxime (HMPAO; Fig. 1B, (*9*)) and a non-nitroimidazole analogue of BMS181321 (6-methyl propyleneamine oxime; PAO-6-Me, Fig. 1C) were used to compare its kinetics with that of BMS181321.

METHODS

Heart Preparations

Female rats (240–300 g of body weight) were anesthetized with thiopental sodium (38 mg/kg intraperitoneally) (Tanabe Seiyaku, Osaka, Japan) and heparinized. After rapid excision of the heart, the aorta was cannulated and retrogradely perfused with a solution of the following composition (in mmole/liters): NaCl 123, KCl 5, $MgSO_4$ 1, HEPES 5, $CaCl_2$ 1.5, Na-acetate 5 and glucose 6, equilibrated with 100% O_2. The pH of the perfusate was adjusted to 7.4 at 37°C by adding NaOH. The heart spontaneously beat at 2.5–3.5 Hz; if the heart rate became slower than 2.5 Hz, the rate was maintained by right ventricular pacing. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve and connected to a pressure transducer (P23XL, Viggo-Spectramed, Oxnard, CA). The balloon volume was set to achieve an initial end-diastolic pressure of 4–10 mmHg, and then volume was kept constant throughout the experiment. Left ventricular pressure and perfusion pressure were recorded with a direct-writing recorder (Gould, Valley View, OH). After 20–30 min of stabilization, the coronary flow rate, as controlled by a peristaltic pump, was adjusted such that the perfusion pressure equalled ~70 mmHg (flow rate; 12.0 ± 0.7 ml/min/g heart weight; Table 1). Once adjusted, the flow rate was kept constant throughout the experiment except when ischemia was induced by stopping the pump. By definition, the developed pressure was determined as the difference between peak left ventricular pressure and end-diastolic pressure. The research protocol was approved by the Animal Care and Use Committee of the institution and animal experiments were performed according to the guidelines of the American Physiological Society.

After stabilization, hearts were set in a glass chamber.

Received Jan. 12, 1994; revision accepted Mar. 31, 1994.

For correspondence and reprints contact: Hideo Kusuoka, MD, PhD, Div. of Tracer Kinetics, Biomedical Research Center, Osaka University Medical School, 2-2 Yamada Oka, Suita Osaka 565 Japan.

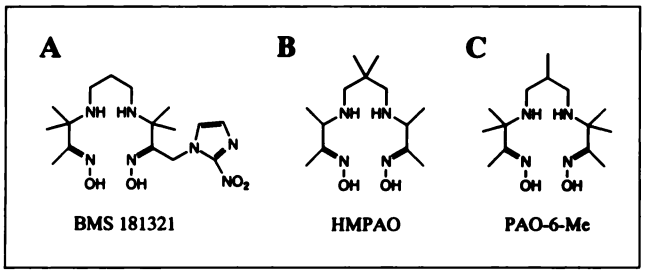

FIGURE 1. Structures of (A) propyleneamine oxime-1,2-nitroimidazole; (B) hexamethyl propyleneamine oxime; and (C) 6-methyl propyleneamine oxime.

BMS181321 (0.1 ~0.5 mCi; Bristol Myers Squibb, Princeton, NJ) was injected into a perfusion line just above the cannula connected to the aorta. Thus, there was no significant time delay between the injection and the perfusion of myocardium by BMS181321. The time-activity curve of BMS181321 uptake in the myocardium was measured with a 1 × 1-inch NaI(Tl) scintillation detector (Steffi, Raytest, Straubenhardt, Germany) positioned at 4 cm from the heart and fitted with an 8-mm thick lead collimator that was 6 cm in length. Count rate data were recorded using a D-2500 Chromato Integrator (Hitachi, Tokyo, Japan). The effluent from the myocardium was collected and not recirculated. At the end of each experiment, the radioactivity in the effluent and the myocardium was measured by a single-channel analyzer with a 2 × 2-inch NaI(Tl) scintillator (Ohyo Koken Kogyo, Tokyo, Japan); the uptake ratio of an agent by the myocardium was expressed as a percent of the injected dose (%ID).

Technetium-99m-HMPAO (0.1 mCi; Amersham International, London) or ^{99m}Tc-PAO-6-Me (0.5 mCi; Bristol Myers Squibb, Princeton, NJ) was used in some protocols instead of BMS181321.

Preparation of BMS181321

Synthesis of BMS181321 has been described previously (*6*). BMS181321 was prepared from provided kits as indicated. Briefly, sodium pertechnetate in normal saline was added to the ligand vial. The stannous DTPA vial containing 10 mg pentatate calcium trisodium and 0.5 mg $SnCl_2 \cdot 2H_2O$ under a nitrogen atmosphere was reconstituted with 4 ml of normal saline, a 0.15-ml aliquot was removed and added to the vial containing ligand and pertechnetate. After 10 min at room temperature, formation of BMS181321 was completed. The radiochemical purity of the ^{99m}Tc complexes was 90% as determined by high-pressure liquid chromatography using a Hamilton PRP-1 column eluted with acetonitrile/0.1 *M* NH_4OAc, pH 4.6, or by the paper chromatography using Gelman solvent saturation pad developed in diethyl ether.

Experimental Protocol

Ischemia/Reperfusion Experiments. After stabilization of the preparation, hemodynamic parameters before ischemia were measured. Then, hearts were subjected to 15 min of global ischemia at 37°C, and reperfused. To make global ischemia, coronary flow was decreased to 0 ml/min by stopping the pump and cross-clamping the perfusion line. Hemodynamics were evaluated again 10–20 min after the start of the reperfusion. BMS181321 was injected before or after ischemia as indicated below, and the time-activity curve was obtained (Fig. 2).

In the first protocol, BMS181321 was injected into the perfusate during normoxic perfusion. The activity in the myocardium was measured for 10 min after the injection. These data served as the control. Five hearts were dedicated to this protocol.

In the second protocol, BMS181321 was injected into the perfusate 30 sec *before* global ischemia in two hearts. After 15 min of ischemia, hearts were reperfused for 10 min. The activity in the myocardium was measured for 25 min after the injection.

In the third protocol, hearts were first subjected to global ischemia for 15 min before being reperfused to produce stunned myocardium (*10*). BMS181321 was injected at the start of reperfusion in two hearts. Reperfusion continued for 10 min. The activity in the myocardium was obtained for 10 min.

In the fourth protocol, hearts were stunned by 15 min of global ischemia and reperfusion. BMS181321 was given 10 min after the start of reperfusion. Hearts were reperfused for an additional 10 min, i.e., a total of 20 min. The activity in the myocardium was measured for 10 min. This protocol was applied to two hearts.

Hypoxia Experiments. After stabilization, cardiac function was evaluated before hypoxia. Then, perfusate bubbled with 100% O_2 was switched to that saturated with 100% N_2 (Fig. 2). Hearts reached a new steady state within 10 min after switching the

TABLE 1
Hemodynamic Parameters in the Various Experimental Groups

	Peak p	EDP	DP
Left ventricular function			
Normoxic control group (n = 8)			
	106.8 ± 3.7	5.9 ± 1.1	101.0 ± 3.3 mmHg
Stunned group (n = 9)			
Before ischemia	107.8 ± 3.2	5.1 ± 1.2	102.7 ± 3.6 mmHg
After stunning	94.2 ± 7.7	10.8 ± 3.4	83.3 ± 10.4 mmHg
Hypoxic group (n = 6)			
During normoxia	98.0 ± 3.0	6.8 ± 1.6	91.2 ± 4.2 mmHg
During hypoxia	43.4 ± 12.5	11.2 ± 1.0	32.2 ± 12.7 mmHg
Coronary flow rate			
Normoxic control group	13.8 ± 1.5 ml/min/g wet wt		
Stunned group	10.5 ± 0.4		
Hypoxic group	11.5 ± 0.7		
Pooled data	12.0 ± 0.7		

Peak p = peak left ventricular pressure; EDP = end-diastolic pressure; and DP = developed pressure.

perfusate. Cardiac function was evaluated again during hypoxia. BMS181321 was added to the perfusate, and the activity in the myocardium was measured for 10 min after the injection. Four hearts were dedicated to the hypoxic experiments.

HMPAO or PAO-6-Me was used as a substitute for BMS181321 in part of the protocols above in order to compare their kinetics to BMS181321. Five hearts were used in the experiments with HMPAO and three were used for PAO-6-Me.

Statistical Analysis

Data are presented as the mean ± s.e.m. Statistical analysis was performed using the paired t-test or analysis of variance (ANOVA) where appropriate. Probability of the null hypothesis of $p < 0.05$ was considered significant.

RESULTS

Kinetics of BMS181321 in Normoxic Myocardium

Left ventricular function in the normoxic control group is summarized in Table 1. Figure 3A shows the time-activity curve of BMS181321 in a normoxic heart. The counts in the myocardium were normalized to the maximum value at 10 sec after the injection. BMS181321 was quickly washed out, and the retention at 10 min after the injection was only 1.1% ID/g wet wt. Such a rapid washout was consistently observed in five hearts. The retention in the myocardium 10 min after the injection was 0.84% ± 0.04% ID/g wet wt (Fig. 4).

Kinetics of BMS181321 in Stunned Myocardium

In stunned myocardium, cardiac function decreased significantly (Table 1). Peak left ventricular pressure slightly decreased (107.8 ± 3.2 mmHg in control, 94.2 ± 7.7 mmHg after stunning; $0.10 > p > 0.05$, $n = 7$), and end-diastolic pressure significantly increased from 5.1 ± 1.2 mmHg to 10.8 ± 3.4 mmHg ($p < 0.05$); the developed pressure in stunned myocardium (83.3 ± 10.4 mmHg) significantly decreased compared with that before ischemia (102.7 ± 3.6 mmHg, $p < 0.05$).

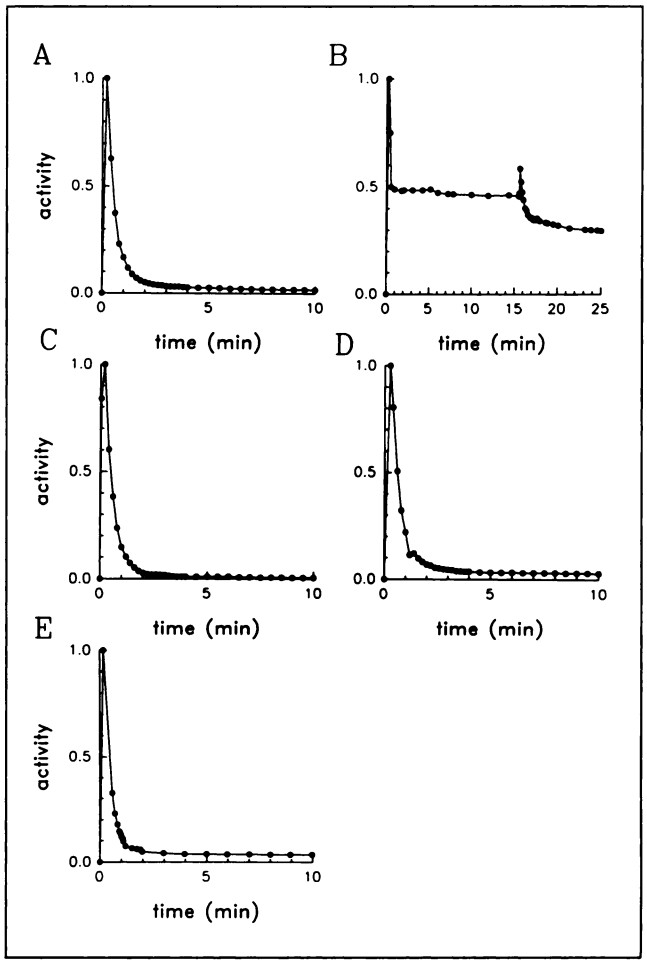

FIGURE 3. The time-activity curves of BMS181321 in isolated perfused rat hearts. (A) Normoxic heart. (B) BMS181321 was injected 30 sec before ischemia. A heart was subjected to 15-min global ischemia and reperfused for 10 min. (C) BMS181321 was injected at the start of reperfusion after 15-min global ischemia. (D) BMS181321 was injected 10 min after reperfusion. (E) Hypoxic heart.

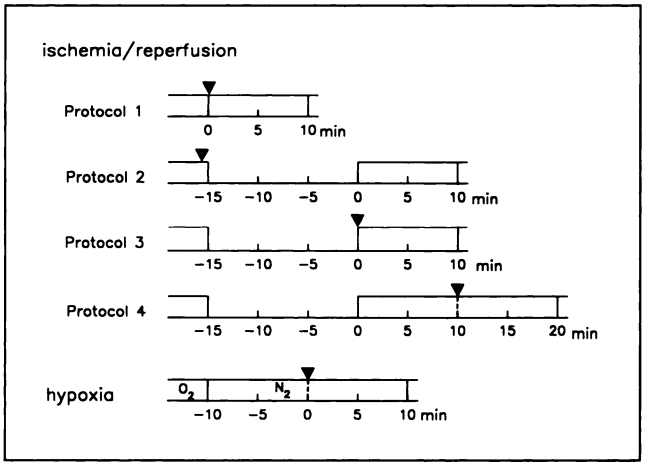

FIGURE 2. Experimental protocol. ▼ indicates the injection of radioactive ligand. Protocol 1: normoxic perfusion. Protocol 2: injection 30 sec before global ischemia. Protocol 3: injection at the start of reperfusion. Protocol 4: injection 10 min after the start of reperfusion. Hypoxia: injection 10 min after switching to hypoxia.

Panels B–D in Figure 3 show representative time-activity curves of BMS181321 in hearts subjected to ischemia and reperfusion. When BMS181321 was injected before ischemia, it was trapped not only during no flow ischemia but also retained after reperfusion (Fig. 3B). Reperfusion washed out only 37% of BMS181321 remaining in the myocardium; the uptake in myocardium 10 min after reperfusion was 18.4% ID/g wet wt of the administered dose, which was 63% of the dose retained by the cessation of flow. In contrast, the behavior of BMS181321 in stunned myocardium was similar to that in the control, when the agent was injected after reperfusion. Panels C and D in Figure 3 show the time-activity curves in stunned myocardium where BMS181321 was injected at the start of reperfusion (Panel C) or 10 min after the start of reperfusion (Panel D), respectively. The retention of BMS181321 in myocardium 10 min after the injection is summarized in Figure 4. The retention in ischemic myocardium was significantly greater than in the control (23.9% ± 3.9% ID/g

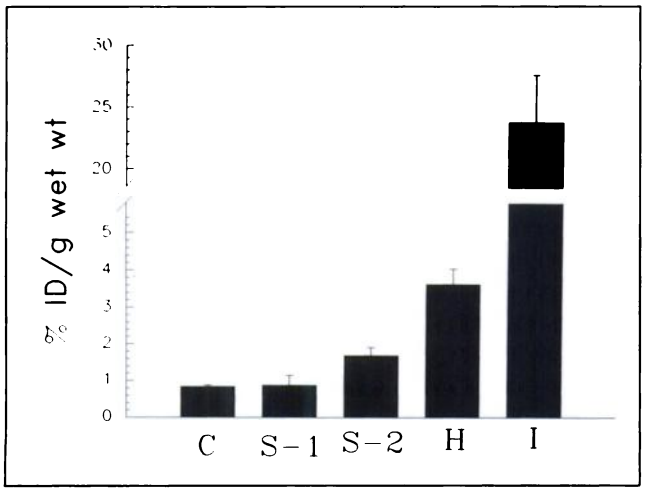

FIGURE 4. The myocardial retention of BMS181321 at the end of each experiment. C = Normoxic control (n = 5). S-1 = BMS181321 was injected at the start of reperfusion (n = 2). S-2 = BMS181321 was injected at 10 min after reperfusion (n = 2). H = Hypoxic condition (n = 4). I = BMS181321 was injected at 30 sec before ischemia (n = 2).

wet wt, $p < 0.05$), but that in stunned myocardium was identical to the control.

Kinetics of BMS181321 in Hypoxic Myocardium

When a perfusate was switched to a hypoxic one, hearts rapidly reduced their contractility and reached a new steady state within 5 min (Table 1). End-diastolic pressure increased from 6.8 ± 1.6 mmHg to 11.2 ± 1.0 mmHg (n = 6; $p < 0.05$), and developed pressure decreased from 91.2 ± 4.2 mmHg to 32.2 ± 12.7 mmHg (35.3% of control value; $p < 0.01$).

Figure 3E shows the time-activity curve of BMS181321 in hypoxic myocardium. The initial change within 1 min after the injection was similar to that in normoxic control myocardium (Fig. 3A). However, the washout was slowed during the late phase compared to normoxia. The retention at 10 min after injection was 3.4% ID/g wet wt in this hypoxic heart. Such a change was observed consistently in four experiments. The retention at 10 min after injection (3.63% ± 0.42% ID/g wet wt; Fig. 4) was about fourfold higher than that in normoxic control hearts ($p < 0.05$).

Kinetics of HMPAO in Normoxic, Hypoxic and Ischemic Myocardium

To compare the kinetics of BMS181321 to that of HMPAO, HMPAO was injected into normoxic, hypoxic or ischemic myocardium. The time-activity curves in each experiment are shown in Figure 5. The experimental protocol was identical to that of BMS181321. HMPAO was highly retained even in normoxic hearts; the time-activity curve of HMPAO in the normoxic heart (Fig. 5A) was quite similar to that of BMS181321 in the ischemic heart (Fig. 5B). The time-activity curves of HMPAO in hypoxic and ischemic myocardium (Figs. 5B and C) were almost identical to that in the normoxic heart. The retention at the end of each experiment in normoxic, hypoxic or ischemic hearts is summarized in Figure 6. The retention of HMPAO is not significantly different among normoxic, hypoxic and ischemic myocardium ($p > 0.50$).

Kinetics of PAO-6-Me in Normoxic and Ischemic Myocardium

To compare the kinetics of BMS181321 to that of its non-nitroimidazole analogue, PAO-6-Me was injected into normoxic or ischemic myocardium. The time-activity curves in each experiment are shown in Figure 7. The experimental protocol was identical to that of BMS181321. PAO-6-Me was quickly washed out from the myocardium in normoxia; the time-activity curve of PAO-6-Me (Fig. 7A) and that of BMS181321 (Fig. 3A) in the normoxic heart were very similar. The myocardial retention of PAO-6-Me 10 min after injection was 0.5% ID/g wet wt.

The time-activity curve of PAO-6-Me injected 30 sec before ischemia (Fig. 7B) was markedly different from those of BMS181321 (Fig. 3B) and HMPAO (Fig. 5B). As with BMS181321 and HMPAO, PAO-6-Me was trapped during no flow ischemia. However, in contrast to either BMS181321 or HMPAO, PAO-6-Me was quickly washed out after reperfusion. The retention of PAO-6-Me at the end of the experiment in ischemic myocardium was only 3.25% ± 0.32% ID/g wet wt (n = 2), and lower than that of BMS181321 or HMPAO ($0.10 > p > 0.05$).

DISCUSSION

Our results indicate that 63% of the BMS181321 trapped in the myocardium by no flow ischemia is retained in contrast to less than 2% retained when injected after reperfusion (stunned myocardium). Hypoxic myocardium also

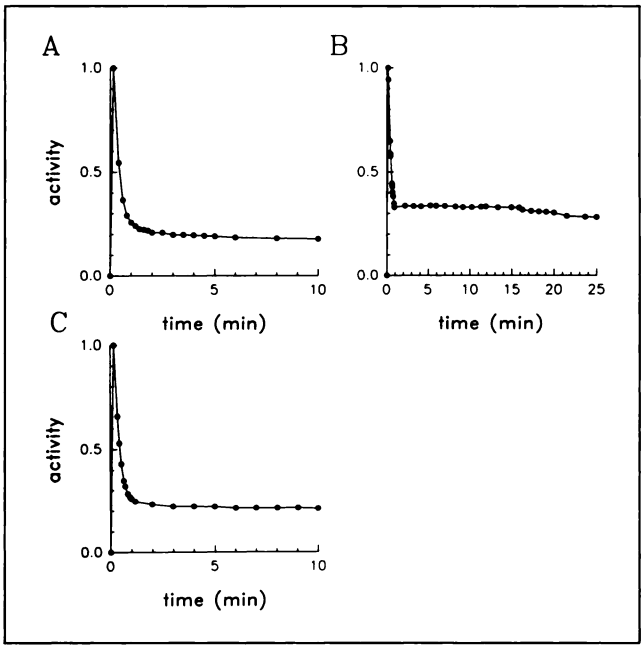

FIGURE 5. The time-activity curves of HMPAO in isolated perfused rat hearts. (A) Normoxic heart. (B) HMPAO was injected at 30 sec before ischemia. A heart was subjected to 15-min global ischemia and reperfused for 10 min. (C) Hypoxic heart.

shows significantly higher retention at 10 min after the injection (about fourfold) compared to normoxic myocardium. These observations are consistent with the kinetics of BMS181321 in in vivo hearts (*11,12*).

It is well recognized that 15 min of global ischemia is not sufficient to cause necrosis, however, it is sufficient to impair contractile function after reperfusion. Myocardium impaired in this manner is known as stunned myocardium (*8*). Myocardium is rapidly reoxygenated and energy metabolism returns to the normoxic pattern within 5 min when a heart is reperfused after a brief period of ischemia (*10*). Thus, it is not surprising that the kinetics of BMS181321 in stunned myocardium were similar to that in normoxia when BMS181321 was injected after reperfusion. These results also suggest that the time window of anoxia or hypoxia, during which BMS181321 may markedly be retained by myocardium, exceeds 5 min.

The kinetics of HMPAO and PAO-6-Me suggest the mechanism for trapping BMS181321 in ischemic myocardium. Ischemic myocardium retained an extremely high amount of BMS181321 when injected just before an ischemic insult (23.9% ID/g wet wt, or 63% of hydrodynamically-trapped material resident for 15 min), but hypoxic myocardium retained only a small amount (4.2% ID/g wet wt). These results suggest two possible mechanisms for the trapping of BMS181321 in ischemic myocardium. One is that BMS181321 is trapped by metabolism as is believed for the trapping of HMPAO in brain (*9*), and the other is that the nitroimidazole portion of the compound reflects the difference.

Although the mechanism for trapping HMPAO by myocardium is unresolved, our results showed that the retention of HMPAO in hypoxic myocardium did not differ from

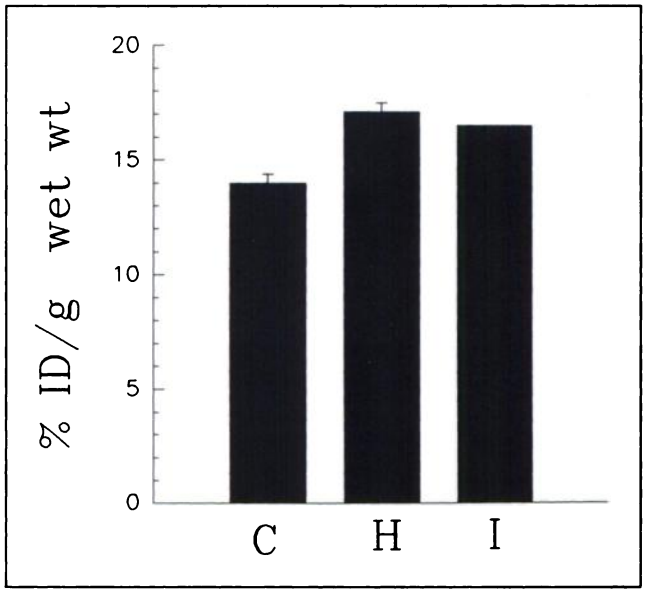

FIGURE 6. The myocardial retention of HMPAO at the end of each experiment. C = normoxic control (n = 2). H = hypoxic condition (n = 2). I = HMPAO injected 30 sec before induction of ischemia (n = 1).

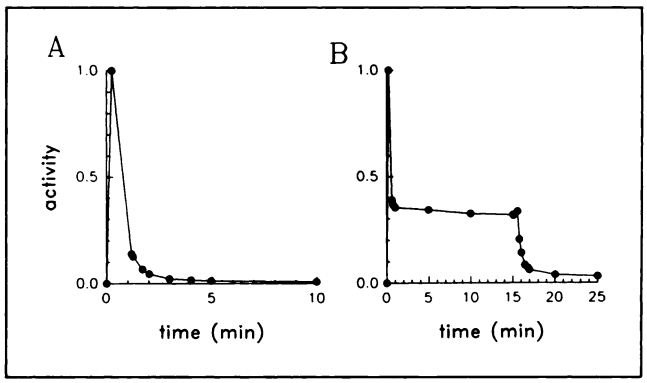

FIGURE 7. The time-activity curves of PAO-6-Me in isolated perfused rat hearts. (A) Normoxic heart. (B) PAO-6-Me was injected 30 sec before ischemia was induced. The heart was subjected to 15 min of global ischemia and then reperfused for 10 min.

that in the ischemic one. The metabolic change during ischemia is more severe than that during hypoxia; ischemia induces severe acidosis and a marked increase of inorganic phosphate compared to hypoxia (*10,13*). These results suggest that severe anoxia may alter the structure of BMS181321 and increase its retention after reperfusion. However, the nonimidazole analogue of BMS181321, PAO-6-Me, is quickly washed out during reperfusion after ischemia. Such a quick washout was also reported in hypoxic myocardium for this compound (*14*). Thus, these results indicate that the activity for BMS181321 is conferred by the nitroimidazole group, and that the difference presumably reflects the different residence times of BMS181321 in the myocardium, i.e., 15 min for no flow ischemia versus single pass for hypoxia.

The applicability of BMS181321 to the clinical use is one of the most interesting questions. The present study indicates that BMS181321 can clearly image an ischemic myocardium if it is injected before ischemia, but not in stunned myocardium. These results suggest that BMS181321 is useful to detect ischemia induced by exercise because the agent can be applied before the onset of ischemia. In contrast, a fourfold increase of uptake by hypoxic myocardium might not be sufficient to image a hypoxic myocardium because of its high uptake in the liver (*14*). Thus, further in vivo studies are necessary to clarify the clinical applicability of BMS181321.

ACKNOWLEDGMENT

The authors thank Bristol-Myers Squibb Pharmaceutical Research Institute for their kind gift of BMS181321.

REFERENCES

1. Chapman JD. Hypoxic sensitizers—implications for radiation therapy. *N Eng J Med* 1979;301:1429–1432.
2. Shelton ME, Dence CS, Hwang D-R, Welch MJ, Bergmann SR. Myocardial kinetics of fluorine-18 misonidazole: a marker of hypoxic myocardium. *J Nucl Med* 1989;30:351–358.
3. Shelton ME, Dence CS, Hwang D-R, Herrero P, Welch MJ, Bergmann SR. In vivo delineation of myocardial hypoxia during coronary occlusion using fluorine-18-fluoromisonidazole and positron emission tomography: a poten-

tial approach for identification of jeopardized myocardium. *J Am Coll Cardiol* 1990;16:477–485.
4. Martin GV, Caldwell JH, Graham MM, et al. Noninvasive detection of hypoxic myocardium using fluorine-18-fluoromisonidazole and positron emission tomography. *J Nucl Med* 1992;33:2202–2208.
5. Martin GV, Biskupiak JE, Caldwell JH, Rasey JS, Krohn KA. Characterization of iodovinylmisonidasole as marker for myocardial hypoxia. *J Nucl Med* 1993;34:918–924.
6. Linder KE, Chan YW, Cyr JE, Malley MF, Nowotnik DP, Nunn AD. TcO(PnAO-1-(2-nitroimidazole)) [BMS-181321], a new technetium-containing nitroimidazole complex for imaging hypoxia: synthesis, characterization, and xanthine oxidase-catalyzed reduction. *J Med Chem* 1994;37:9–17.
7. Rumsey WL, Cyr JE, Raju N, Narra RK. A novel technetium-99m-labeled nitroheterocycle capable of identification of hypoxia in heart. *Biochem Biophys Res Commun* 1993;193:1239–1246.
8. Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation* 1982;66:1146–1149.
9. Neirinckx RD, Canning LR, Piper IM, et al. Technetium-99m d,l-HM-PAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J Nucl Med* 1987;28:191–202.
10. Kusuoka H, Porterfield JK, Weisman HF, Weisfeldt ML, Marban E. Pathophysiology and pathogenesis of stunned myocardium: depressed Ca^{2+} activation of contraction as a consequence of reperfusion-induced cellular calcium overload in ferret hearts. *J Clin Invest* 1987;79:950–961.
11. Rumsey WL, Patel B, Kuczynski B, et al. Planar and SPECT of ischemic canine myocardium using a novel ^{99m}Tc-nitroimidazole [Abstract]. *J Nucl Med* 1993;34:15P.
12. Stone CK, Mulnix T, Nickles RJ, et al. Myocardial kinetics of a putative hypoxic tissue marker, technetium-99m-labeled nitroimidazole, (BMS-181321) after regional ischemia and reperfusion [Abstract]. *J Nucl Med* 1993;34:16P.
13. Marban E, Kusuoka H. Maximal Ca^{2+}-activated force and myofilament Ca^{2+} sensitivity in intact mammalian hearts. Differential effects of inorganic phosphate and hydrogen ions. *J Gen Physiol* 1987;90:609–623.
14. Rumsey WL, Cyr JE, Raju N, et al. A novel technetium-99m-labeled nitroheterocycle capable of identification of hypoxia in heart: in vitro and tissue distribution studies [Abstract]. *J Nucl Med* 1993;34:146P.