Myocardial Kinetics of Fluorine-18 Misonidazole: A Marker of Hypoxic Myocardium

Marc E. Shelton, Carmen S. Dence, D.-R. Hwang, Michael J. Welch, and Steven R. Bergmann

Cardiovascular Division, Department of Internal Medicine and Division of Radiation Sciences, Washington University School of Medicine, St. Louis, Missouri

Fluoromisonidazole, a member of a class of compounds referred to as "hypoxic sensitizers," accumulates in hypoxic, viable tumor cells. We hypothesized that it might therefore accumulate also in ischemic, but non-necrotic myocardium potentially salvageable by interventional therapy. To evaluate the myocardial kinetics of [^{18}F]fluoromisonidazole (FM), 20 isolated perfused rabbit hearts were used to characterize the uptake and binding of tracer under control conditions (n = 6), or with ischemia (flow 10% of control, n = 5), hypoxia without low flow (control flow rates with hypoxic medium, n = 5), or with reperfusion (n = 4). Myocardial retention of tracer detected externally with gamma scintillation probes after 20 min of constant [^{18}F]FM infusion followed by 20 min of washout with nonradioactive buffer was 41 ± 7% and 46 ± 8% of peak activity in hearts subjected to ischemia or hypoxia, respectively, and significantly higher than in hearts subjected to either control perfusion or to ischemia followed by reperfusion (18 ± 6 and 16 ± 5% of peak activity, respectively, $p < 0.01$). The biologic half-time of retained tracer was 40 hr in all hearts indicating essentially irreversible binding. Based on these findings, we measured uptake of [^{18}F]FM using positron emission tomography in five dogs subjected to acute coronary occlusion. Five to thirteen millicuries of tracer were injected within 3 hr of occlusion. Within 30 min after administration of tracer, ^{18}F accumulation in ischemic myocardium was greater than that observed in normal myocardium. The results indicate that [^{18}F]FM accumulates in ischemic myocardium in relation to diminished tissue oxygen content and not simply because of diminished flow. Thus, this class of compounds may be potentially useful to help identify hypoxic myocardium.

J Nucl Med 30:351–358, 1989

The etiology of chest pain, potentially indicative of ischemia is often unclear. Its delineation is essential in part because interventions such as thrombolysis salvages myocardium jeopardized by acute coronary thrombotic occlusions only with early administration after the onset of ischemia (*1*). Currently there is no sensitive, noninvasive diagnostic test that can rapidly identify or quantify jeopardized (i.e., ischemic or hypoxic but not yet necrotic) myocardium (*2*).

We previously demonstrated that positron emission tomography with oxygen-15- (^{15}O) labeled water can be used to quantify myocardial perfusion (*3*). However, during acute coronary events, estimation of areas of diminished blood flow, although defining areas-at-risk, does not permit differentiation between jeopardized and nonviable myocardium. Positron-emitting radiotracers such as fluorine-18 fluorodeoxyglucose [^{18}F]FDG (*4*) or rubidium-82 (^{82}Rb) (*5*) have been proposed for use in identification of jeopardized myocardium, but the complex relationship between blood flow and metabolism and the dependence of uptake and retention of [^{18}F]FDG or ^{82}Rb on factors such as circulating levels of substrate and hormones and on the duration of ischemia have complicated the use of these tracers for identification of jeopardized myocardium (*2,6–9*).

Although we recently demonstrated that carbon-11 (^{11}C) acetate is a valid tracer of myocardial oxygen consumption (*10,11*), after transient ischemia, myocardial oxygen consumption continues to be depressed and may not recover for 24 hr or more (*12*).

Received July 11, 1988; revision accepted Oct. 11, 1988.

For reprints contact: Steven R. Bergmann, MD, PhD, Cardiovascular Division, Washington University School of Medicine, Box 8086, 660 S. Euclid Ave., St. Louis, MO 63110.

The nitroimidazoles are a class of compounds with high electron affinity that have been studied extensively as radiosensitizers of hypoxic regions in tumors (*13*). The most extensively studied radiosensitizer, 1-(2-nitro-1-imidazolyl)-3-methyoxy-2-propanol (misonidazole), has been shown to accumulate in hypoxic regions of multicellular spheroids and EMT6 tumors with little uptake into the necrotic core (*14,15*). These results suggest that labeled congeners of misonidazole might serve as novel imaging agents for non-necrotic, reversibly hypoxic tissue. A fluorinated derivative, fluoromisonidazole, labeled with the positron emitting radionuclide ^{18}F was first synthesized by Jerabek et al. (*16*) and subsequently shown to exhibit increased uptake in ischemic brain tissue (*17*). Tritiated fluoromisonidazole has been shown in preliminary reports to have increased uptake in hypoxic isolated myocytes (*18*) and in intact ischemic canine myocardium (*19*).

The aim of the present study was to characterize the accumulation and retention of ^{18}F fluoromisonidazole ([^{18}F]FM) in an isolated perfused rabbit heart preparation in which the variables of tracer delivery, myocardial perfusion, and perfusate oxygenation could be controlled to determine whether this radiopharmaceutical would be useful for identification of hypoxic myocardium.

METHODS

Isolated Heart Preparation

Male New Zealand rabbits weighing 1.5 kg to 2 kg were stunned with a blow to the head. Hearts were excised rapidly and perfused retrogradely via the aorta at 37°C with nonrecirculating, oxygenated (95% O_2/5% CO_2) modified Krebs-Henseleit perfusate containing 0.4 m*M* albumin, 5 m*M* glucose, and 70 mU/l insulin (*20*). Hearts were paced at 180 bpm with a right atrial bipolar electrode. Left ventricular pressure was monitored continuously with a fluid-filled latex balloon inserted through the left atrium. Left ventricular end-diastolic pressure was set at 5–10 mmHg by adding volume to the ventricular balloon. The first derivative of left ventricular pressure (dP/dt) and coronary perfusion pressure were also monitored continuously. The pulmonary artery was cannulated for collection of coronary venous effluent. Oxygen content of the perfusate was measured with a co-oximeter. Paired external sodium iodide gamma scintillation crystals were oriented at 180° across the heart for detection of radioactivity (Fig. 1). Coincidence counts were detected with an Ortec fast coincidence counter and recorded with a Digital Equipment Corporation RX-08 minicomputer. Data were stored on floppy disks for subsequent analysis.

Experimental Procedure

All hearts were perfused initially under control conditions (i.e., with oxygenated buffer at a control flow rate of 20 ml/min; ~4 ml/g/min-supraphysiologic but necessary for adequate oxygen delivery with crystalloid buffer). After stabilization of left ventricular pressure, dP/dt, left ventricular end-diastolic pressure, and perfusion pressure (at least 15 min), the kinetics of [^{18}F]FM were evaluated in each heart over 40 min. Hearts from four groups were studied. A control group (n = 6) was evaluated to characterize [^{18}F]FM accumulation and retention in normal myocardium. In these hearts, perfusate flow and oxygenation was not altered over the experimental period. Accumulation of tracer in the heart was monitored during 20 min of infusion of [^{18}F]FM, accomplished by rapidly switching the parallel circuits of the perfusion system to the one containing buffer with [^{18}F]FM at a concentration of ~1 μCi/ml. After the 20-min period of tracer infusion, the hearts were then perfused with non-radioactive (or "cold") buffer for an additional 20-min observation period so that retention of tracer in the myocardium could by monitored. To evaluate the effects of ischemia, the accumulation of [^{18}F]FM was

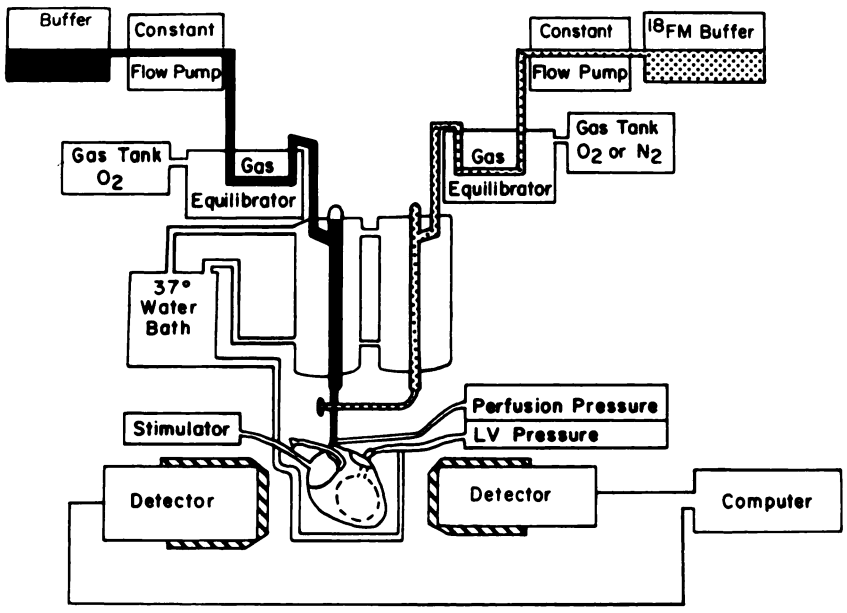

FIGURE 1
Schematic diagram of the isolated perfused heart preparation used in these studies. The nonrecirculating system permitted rapid switching from buffer containing tracer to non-radioactive perfusate. Two gamma scintillation crystals were placed at 180° across the heart for detection of coincident events (see text for details).

monitored during low flow induced by reducing perfusate flow rate to 10% of control (i.e., 2 ml/min of oxygenated perfusate, n = 5). After 20 min of infusion of tracer, radioactivity was monitored during 15 min of washout with "cold" buffer at the ischemic flow rate. After 15 min of washout, flow was increased to control flow rates to evaluate the effects of bulk flow in this group of hearts. A third group of hearts (the hypoxic group, n = 5) was studied to evaluate the effects of hypoxia per se. In this group, after the equilibration period, hearts were subjected to hypoxia, achieved by equilibrating the perfusate containing tracer with 95% nitrogen/5% CO_2 which diminished perfusate pO_2 to <40 mmHg while maintaining pH and pCO_2 at the physiologic level. Radioactivity was monitored for 20 min during tracer infusion, and during washout with "cold" oxygenated buffer at control flow rate for an additional 20 min.

To evaluate the effects of reperfusion and reoxygenation on the myocardial handling of [^{18}F]FM, a group of hearts were studied during reperfusion. These hearts (n = 4) were subjected to 35 min of ischemic flow followed by 20 min of reperfusion at control flow rates with oxygenated buffer. This protocol was selected based on results of previous studies from our laboratory which showed that this period of ischemia and reperfusion resulted in recovery of ventricular function to >90% of pre-ischemic values. Twenty minutes after the initiation of reperfusion, hearts were then subjected to tracer infusion for 20 min followed by 20 min of washout at control flow rates.

In selected hearts, perfusate and coronary venous radioactivity were monitored directly in a gamma well counter for direct estimates of tracer extraction.

Analysis of Time-Activity Curves

As depicted in Figure 2, which shows representative myocardial time-activity data, radioactivity accumulated in isolated hearts over the period of tracer infusion. Washout appeared to be biexponential with a rapid phase, presumably washout of tracer from vascular and interstitial pools, and a much more prolonged phase thought to represent irreversibly bound tracer. For analysis, radioactivity at the end of the washout period was compared to peak radioactivity (just prior to switching to "cold" washout), and expressed as the residual fraction (i.e., the percentage of radioactivity remaining in the hearts compared with peak radioactivity). Estimates of the biologic half-time of tracer were made by a least squares fit of the decay corrected time-activity data during the last 15 min of the washout period after washout of tracer from the vasculature.

Synthesis of No-Carrier-Added 3-[^{18}F]-Fluoro-1-(2-Nitro-1-Imidazolyl)-2-Propanol

No-carrier-added [^{18}F]FM was synthesized by the recently reported method of Hwang et al. (*21*). Briefly, 200 mCi of ^{18}F in $H_2{}^{18}O$ was mixed with 2 mg of K_2CO_3 and 6 mg of Kryptofix 222, heated at 105–110° under N_2 and azeotropically dried with CH_3CN. The residue was taken up in 200 μl of anhydrous CH_3CN, transferred to a 1-ml conical vial containing 4 mg of (2R) (−) glycidyl tosylate and heated for 12 min at 95%. The resulting [^{18}F]epifluorhydrin was isolated by passing the reaction mixture through a short silica column and eluting with CH_3CN (100 μl). The solution of epifluorohydrin was added to a 1-ml conical vial containing 2 mg of 2-nitroimidazole, 6 mg of $NaHCO_3$, and 300 μl of H_2O. The mixture was heated with stirring for 45 min at 95%. The product was purified by preparative high performance liquid chromatography (HPLC) on a silica column using CH_2Cl_2/CH_3CN 55:45 (v/v) as the mobile phase at 1.5 ml/min. The overall synthesis time was 2 hr. Radiochemical purity, determined by HPLC was consistently 99%, and estimated specific

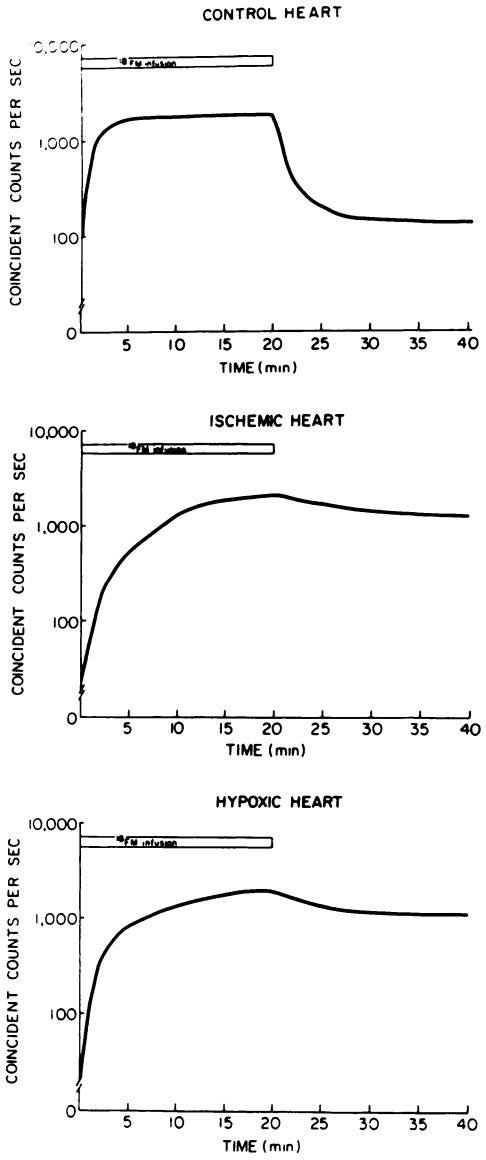

FIGURE 2
Representative time-activity curves from three hearts. The horizontal bar in each graph represents the time of infusion of a constant concentration of [^{18}F]FM. Control hearts were perfused at 20 ml/min throughout the observation period. Ischemic hearts were subjected to a flow of 10% of control with oxygenated media. Hearts subjected to hypoxia were perfused at control flow rates but with hypoxic medium (pO_2 < 40 mmHg) during the tracer infusion period, and with oxygenated medium (pO_2 > 450 mmHg) during the washout phase. Hearts subjected to ischemia or hypoxia accumulated and retained significantly more tracer than hearts subjected to control perfusion or hearts subjected to reperfusion.

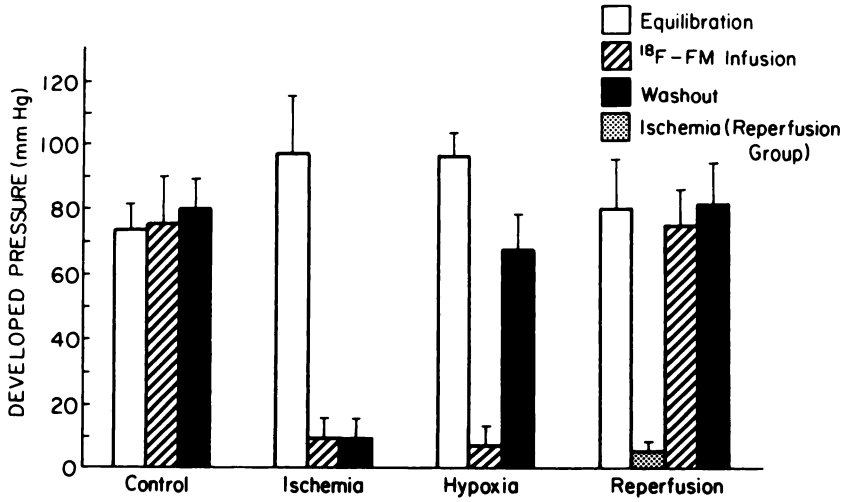

FIGURE 3
Histogram summarizing developed pressure in the hearts studied. Depicted is pressure during the equilibration period, during infusion of tracer, and during the washout period. In addition, developed pressure during ischemia prior to reperfusion is shown for hearts subjected to reperfusion. Values indicate the mean ± the standard deviation.

activity was >400 Ci/mmol. The tracer dissolved in normal saline and was radiochemically stable for at least 4 hr at room temperature.

Positron Emission Tomography

The primary objective of our study was to characterize the myocardial kinetics of [^{18}F]FM under controlled conditions in isolated perfused hearts. However, based on the promising findings obtained (see below), we then performed a pilot study to evaluate the feasibility of imaging ischemic myocardium in intact animals with this tracer.

Coronary thrombosis was induced as previously described (*1*) in five closed-chest, anesthetized, mongrel dogs (20–25 kg) by placement of a thrombogenic copper coil into the left anterior descending coronary artery distal to the first diagonal branch. Verification of coronary occlusion was documented by selective angiography after development of ST segment elevation on the electrocardiogram. Animals were then secured in a Plexiglas shell and positioned within the PETT VI tomograph (*22*). To delineate myocardial blood flow, 25–30 mCi of ^{15}O-labeled water were administered intravenously and data collected for 90 sec (*3*). To delineate blood pool, after decay of radioactivity from ^{15}O water, animals inhaled 40–50 mCi of ^{15}O carbon monoxide. After allowing 1 min for radioactivity to clear the lungs, data were collected for 5 min. Ten minutes following the ^{15}O carbon monoxide scan (an average of 2 hr and 45 min following coronary occlusion), a bolus of 5–13 mCi [^{18}F]FM was given i.v. Sequential 5–10 min scans were acquired 7.5, 30, 60, 90, and 120 min after the injection of tracer.

For delineation of the distribution of myocardial blood flow, data obtained with ^{15}O water were corrected for intravascular tracer with subtraction of blood-pool radioactivity as described previously (*3*). Because arterial clearance of [^{18}F]FM was also prolonged, reconstructions of the data from [^{18}F]FM were corrected for blood-pool radioactivity in an analogous fashion.

Statistical Analysis

Data are expressed as mean ± s.d. Comparisons of unpaired samples were subjected to analysis of variance followed by t-tests corrected for the number of comparisons by the Bonferroni method (*23*). Linear regression was calculated by the least squares method. Probabilities < 0.05 were taken to indicate statistically significant differences.

RESULTS

Hemodynamics

Heart rate, perfusion pressure, left ventricular developed pressure, flow, and dP/dt during the pre-intervention equilibration period were similar in all hearts. Hearts in the control group (n = 6) exhibited stable function throughout the experimental period (Fig. 3). Hearts subjected to ischemia (n = 5) exhibited > 90% reduction in left ventricular developed pressure and dP/dt. Hearts subjected to hypoxia (n = 5) exhibited > 94% reduction of developed pressure and dP/dt during hypoxia (perfusate $pO_2 < 40$ mmHg) but recovered to > 70% of pre-hypoxic values during the washout period with oxygenated media. Hearts subjected to ischemia and reperfusion exhibited > 90% reduction of developed pressure during ischemia, but recovered to > 98% of pre-ischemic values with reperfusion (Fig. 3).

Myocardial Uptake and Clearance of [^{18}F]FM

Time-activity curves are shown in Figure 2. In control hearts, infusion of a constant concentration of [^{18}F]FM led to a rapid rise in tracer in the myocardial field of view. During the washout period with "cold" buffer, clearance of radioactivity from the myocardium was biexponential with a rapid phase apparent early, followed by a plateau. The rapid phase most likely represents clearance of radioactivity from vascular and interstitial compartments, whereas the prolonged phase represents tracer irreversibly bound in tissue. In hearts subjected to ischemia or hypoxia, radioactivity accumulated monotonically during infusion of tracer. During the washout phase, much more radioactivity was retained in both of these groups of hearts compared with that observed in control hearts. In hearts subjected to ischemia, augmentation of flow of perfusate from 2

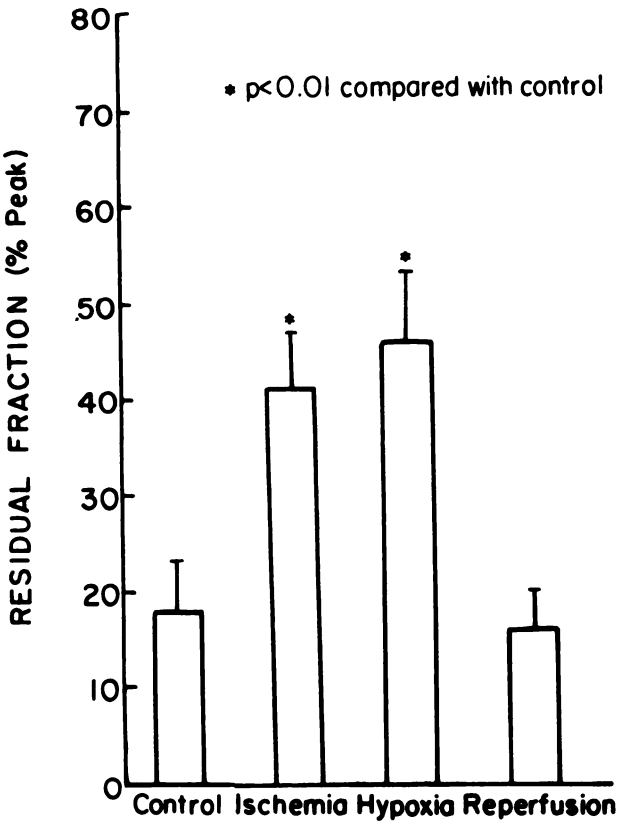

FIGURE 4
Histogram depicting the residual fraction (% of radioactivity retained at the end of the washout period compared to peak radioactivity just prior to the washout period) for all hearts. Data are expressed as the mean ± s.d. Hearts subjected to ischemia or hypoxia retained significantly more tracer than retained in hearts subjected to control perfusion. The enhanced retention was not due simply to bulk flow since hearts subjected to hypoxia were perfused at normal flow rates but with hypoxic media.

TABLE 1
Biologic Half-life of ^{18}F in Isolated Perfused Hearts (hr)

Control (n = 6)	Ischemic (n = 5)	Hypoxic (n = 5)	Reperfusion (n = 4)
66 ± 27	40 ± 6	42 ± 8	83 ± 37

Values are mean ± s.d. Half-life was calculated from the least square regression of data from 5–20 min of the washout period (see text). The data suggest essentially irreversible binding.

ml/min to 20 ml/min during the last 5 min of the washout period did not effect myocardial clearance. Myocardial time-activity curves in hearts subjected to infusion of tracer during reperfusion paralleled those seen in control hearts.

The residual fraction or percent of peak activity retained in the myocardium averaged 18 ± 6% in control hearts, 41 ± 7% in hearts subjected to ischemia, 46 ± 8% in hearts subjected to hypoxia, and 16 ± 5% in hearts subjected to reperfusion (Fig. 4). The difference between hearts subjected to ischemia or hypoxia compared with control hearts was highly significant ($p < 0.01$).

The biologic half-time of tracer in myocardium after 5 min of washout was 40 hr in all hearts studied (Table 1) and consistent with irreversible binding of tracer within the myocardium.

The initial objective of this study was to define the myocardial accumulation and clearance of [^{18}F]FM under diverse conditions in isolated perfused hearts. Because of the promising findings of increased accumulation and prolonged retention in ischemic myocardium, we explored the feasibility of using [^{18}F]FM to delineate jeopardized myocardium by positron emission tomography in canine hearts with regional myocardial ischemia in vivo.

Figure 5 depicts a single mid-ventricular reconstruction from one dog. On the left, the perfusion image depicts a large area of diminished blood flow in the anterior myocardium distal to the occluded left anterior descending coronary artery. Accumulation of [^{18}F]FM (depicted on the right) in the ischemic territory is striking. In all dogs, by 30 min after injection of [^{18}F]FM, ischemic myocardium exhibited enhanced concentrations of tracer despite profound ischemia compared with radioactivity in normal myocardium supplied by the left circumflex coronary artery. The ratio of decay corrected counts/voxel/minute in the anterior compared with normal regions was 1.3 ± 0.2 at 30 min ($p < 0.05$, n = five dogs) and 1.4 ± 0.2 at 60 min ($p < 0.05$, n = 5 dogs) after administration of tracer.

DISCUSSION

Compounds known as hypoxic sensitizers, of which fluoromisonidazole is an example, localize in hypoxic but non-necrotic tumor cells (*13–15*). The results of the present study indicate that fluoromisonidazole also accumulates in ischemic myocardium, an effect not due to low flow per se. Use of this agent may therefore represent a new approach for the identification of hypoxic but potentially salvageable myocardium.

The binding of 2-nitroimidazoles in tumor cell lines and spheroids has been shown to be inversely related to the oxygen content of the media (*24*). Accumulation of tracer was not noted in the necrotic core of the spheroids. Nitroimidazoles are believed to diffuse across the cell membrane and then undergo nitro-reduction in the cytoplasm (*25*). The first step of nitro-reduction occurring, regardless of intracellular pO_2, is the formation of the RNO_2 radical anion. When oxygen is abundant, it reacts with the radical anion yielding superoxide and noncharged misonidazole. Misonidazole then diffuses out of the cell with no net nitro-reduction having

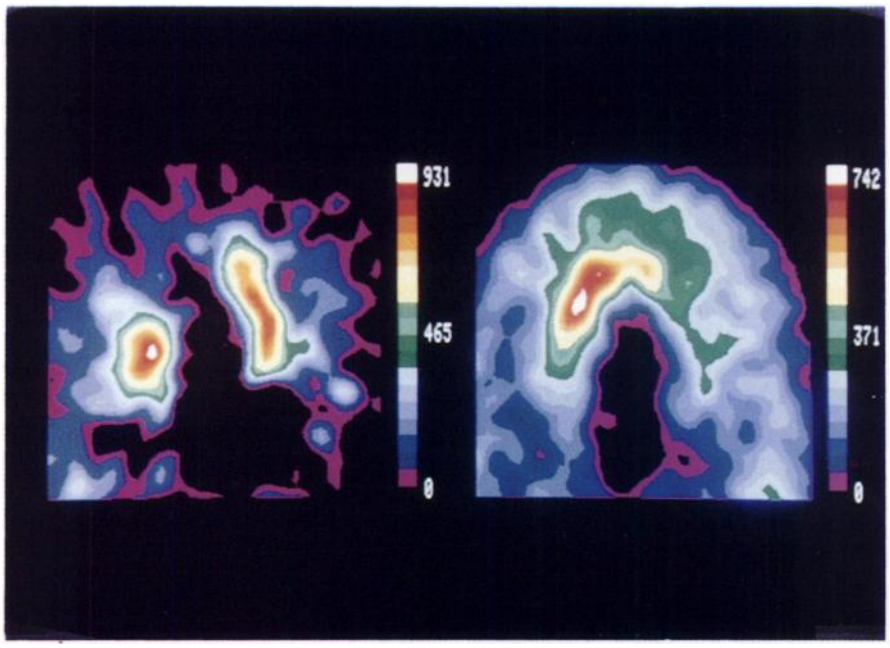

FIGURE 5
A single mid-ventricular slice from one dog subjected to acute coronary occlusion. On the left is the perfusion scan (obtained with ^{15}O-labeled water with correction for blood-pool radioactivity). The septum is to the right, the anterior myocardium is uppermost, and the lateral free wall is to the left. The mitral valve plane is at the bottom. On the perfusion scan, a large flow deficit is observed in the anterior myocardium distal to the territory supplied by the occluded left anterior descending coronary artery. On the right is the concordant tomographic reconstruction obtained 90 min later and 60 min after i.v. administration of 10 mCi of ^{18}F-FM. Marked accumulation is observed in the ischemic region. In all five dogs studied, an enhanced uptake of ^{18}F compared with uptake in normal myocardium was observed in the region of diminished perfusion within 30 min after administration of tracer.

occurred. With hypoxia, reduction of the misonidazole radical anion proceeds further yielding nitroso compounds, and subsequent reduction to hydroxylamines, and other amines may occur. These products can bind covalently to intracellular macromolecules, thereby becoming trapped in the cell. Nitro-reduction in hypoxic cells is believed to be mediated enzymatically in view of its time and temperature dependence (*26*).

Fluorine-18-FM Binding in Myocardium

In control perfused hearts, some tracer was irreversibly trapped in the myocardium, although the residual fraction was only 18%. In contrast, hearts subjected to ischemia or hypoxia showed monotonic increases in accumulation of tracer during the infusion period, and exhibited much more retention of tracer after washout with nonradioactive buffer. Direct perfusate-coronary venous measurements of radiotracer concentration in selected hearts confirmed increased extraction of tracer in ischemic or hypoxic hearts compared with that seen in control hearts [13 ± 12% (n = 2), 2 ± 2% (n = 2) and 1 ± 1% (n = 3), respectively].

To determine whether accumulation and subsequent binding of [^{18}F]FM was attributable to low flow per se (i.e., increased tracer residence), we compared hearts subjected to low-flow ischemia (flow 10% of control) to those perfused at the control flow rate but with hypoxic media (perfusate $pO_2 < 40$ mmHg). The residual fraction and biologic half-times in these two groups were identical indicating that low oxygen and not low flow per se was responsible for the increased accumulation and retention of tracer observed in these hearts. Hearts subjected to hypoxic perfusion were exposed to oxygenated perfusate during the washout period. Since retention was identical to that seen in hearts subjected to ischemia, the results suggest that once the processes that are responsible for irreversible binding have occurred, they cannot be reversed by reoxygenation. This interpretation was confirmed in hearts subjected to ischemia alone since after 15 min of ischemic washout with "cold" buffer, flow rate was increased to control flow rates without change in the content of tracer in the tissue.

To determine the influence of prior ischemia on uptake of tracer, we analyzed a fourth group of hearts subjected to ischemia followed by reperfusion. Tracer was administered after the onset of reperfusion when myocardial function had recovered to pre-ischemic values. The accumulation and retention of tracer in these hearts was identical to control hearts, corroborating the fact that tissue oxygen content is the primary determinant dictating the amount of tracer residualizing in the

myocardium. The prolonged biologic half-times observed in all hearts studied indicate that [^{18}F]FM is bound essentially irreversibly in myocardium (Table 1).

Studies in Intact Animals

In dogs with acute coronary occlusion, myocardial perfusion was delineated with ^{15}O water, and the uptake of [^{18}F]FM by sequential positron emission tomography scanning. Despite profound ischemia induced by coronary occlusion, [^{18}F]FM accumulated in the region of ischemia and by 30 min a clear contrast between ischemic and normal myocardium was definable. In these initial feasibility studies in intact animals, tracer was administered between 2½ and 3 hr after coronary occlusion, an interval that would be expected to result in a mixture of reversibly and irreversibly damaged cells in the ischemic region. We have previously demonstrated that reperfusion initiated after an interval of 2–4 hr is associated with myocardial salvage in this preparation (*1*).

Clinical Implications

The results of the present study indicate that [^{18}F] FM accumulates in hypoxic myocardium by a mechanism independent of flow per se. Because accumulation in ischemic regions in intact dogs permits delineation of hypoxic myocardium within 30 min after tracer administration, this tracer, or other tracers in its class with particularly favorable blood-pool clearance, may permit rapid identification of patients with myocardial hypoxia within the time constraints in which interventions may be salutary.

ACKNOWLEDGMENTS

The authors thank Carla Weinheimer and Howard James for technical assistance, the staff of the Washington University Medical Cyclotron for preparation of the isotopes, and Becky Parrack for preparation of the typescript.

This work was supported in part by NHLBI Grant HL17646, SCOR in Ischemic Heart Disease and NHLBI Grant 13851, Cyclotron Produced Isotopes in Biology and Medicine.

REFERENCES

1 .Bergmann SR, Lerch RA, Fox KAA, et al. Temporal dependence of beneficial effects of coronary thrombolysis characterized by positron tomography. *Am J Med* 1982; 73:573–581.
2. Bergmann SR, Fox KAA, Geltman EM, Sobel BE. Positron emission tomography of the heart. *Prog Cardiovas Dis* 1985; 28:165–194.
3. Bergmann SR, Fox KAA, Rand AL, et al. Quantification of regional myocardial blood flow in vivo with $H_2{}^{15}O$. *Circulation* 1984; 70:724–733.
4. Tillish J, Brunken R, Marshall R, et al. Reversibility of cardiac wall-motion abnormalities predicted by positron tomography. *N Engl J Med* 1986; 314:884–888.
5. Goldstein RA. Kinetics of rubidium-82 after coronary occlusion and reperfusion. *J Clin Invest* 1985; 75:1131–1137.
6. Merhinge ME, Ekas R, Mossberg K, Taegtmeyer H, Gould KL. Catecholamine stimulation, substrate competition, and myocardial glucose uptake in conscious dogs assessed with positron emission tomography. *Circ Res* 1987; 6:II-124–129.
7. Selwyn AP, Allan RM, L'Abbate AL, et al. Relation between regional myocardial uptake of rubidium-82 and perfusion: absolute reduction of cation uptake in ischemia. *Am J Cardiol* 1982; 50:112–121.
8. Schwaiger M, Hansen HW, Sochor H, et al. Delayed recovery of regional glucose metabolism in reperfused canine myocardium demonstrated by positron computed tomography [Abstract]. *J Am Coll Cardiol* 1984; 3:574.
9. Fukuyama T, Nakamura M, Nakagki O. Reduced reflow and diminished uptake of ^{86}Rb after temporary coronary occlusion. *Am J Physiol* 1978; 234:H724–729.
10. Brown MA, Marshall DR, Sobel BE, Bergmann SR. Delineation of myocardial oxygen utilization with carbon-II-labeled acetate. *Circulation* 1987; 76:687–696.
11. Brown MA, Myears DW, Bergmann SR. Noninvasive assessment of canine myocardial oxidative metabolism with ^{11}C-acetate and positron emission tomography. *J Am Coll Cardiol* 1988; 12:1054–1063.
12. Brown MA, Nohara R, Vered Z, Perez JE, Bergmann SR. The dependence of recovery of stunned myocardium on restoration of oxidative metabolism [Abstract]. *Circulation* 1988; 78:II–467.
13. Chapman JD. Hypoxic sensitizers—implications for radiation therapy. *N Engl J Med* 1979; 301:1429–1432.
14. Franko AJ, Chapman JD, Koch CJ. Binding of misonidazole to EMT6 and V79 spheroids. *Int J Rad Oncol Biol Phys* 1982; 8:737–739.
15. Miller GG, Ngan-Lee J, Chapman JD. Intracellular localization of radioactivity labeled misonidazole in EMT-6 tumor cells in vitro. *Int J Rad Oncol Biol Phys* 1982; 8:741–744.
16. Jerabek PA, Patrick TB, Kilbourn MR, Dischino DD, Welch MJ. Synthesis and biodistribution of ^{18}F-labeled fluoronitroimidazoles: potential in vivo markers of hypoxic tissue. *Int J Radiat Appl Instrum* 1986; 37 (part A):599–605.
17. Mathias CJ, Welch MJ, Kilbourn MR, et al. Radiolabeled hypoxic cell sensitizers: tracers for assessment of ischemia. *Life Sci* 1987; 41:199–206.
18. Cerqueira M, Martin G, Embree L, Caldwell J, Krohn K, Rasey J. Enhanced binding of fluoromisonidazole in isolated adult rat myocytes during hypoxia. *J Nucl Med* [Abstract] 1988; 29:807.
19. Martin GV, Rasey JS, Caldwell JC, Grunbaum Z, Krohn KA. Fluoromisonidazole uptake in ischemic canine myocardium [Abstract]. *J Nucl Med* 1987; 28:668.
20. Bergmann SR, Clark RE, Sobel BE. An improved isolated heart preparation for external assessment of myocardial metabolism. *Am J Physiol* 1979; 236: H644–H651.
21. Hwang D-R, Dence CS, Bonasera TA, Welch MJ. No-carrier-added-synthesis of 3-[^{18}F]fluoro-1-(2-nitro-1-imidazolyl)-2-propanol. *J Label Comp Radiopharm* 1988; in press.
22. Ter-Pogossian MM, Ficke DC, Hood JT, Yamamoto

M, Mullani NA. PETT VI: a positron emission tomograph utilizing cessium fluoride scintillation detectors. *J Comp Assist Tomogr* 1982;125–133.
23. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ Res* 1980; 47:1–9.
24. Koch CJ, Stobe CC, Baer KA. Metabolism induced binding of ^{14}C-misonidazole to hypoxic cells: kinetic dependence on oxygen concentration and misonidazole concentration. *Int J Radiat Oncol Biol Phys* 1984; 10:1327–1331.
25. Franko AJ. Misonidazole and other hypoxia markers: metabolism and applications. *Int J Rad Oncol Biol Phys* 1986; 12:1195–1202.
26. Chapman JD, Baer K, Lee J. Characteristics of the metabolism-induced binding of misonidazole to hypoxic mammalian cells. *Cancer Res* 1983; 43:1523–1528.