Enhanced Binding of the Hypoxic Cell Marker [^{3}H]Fluoromisonidazole in Ischemic Myocardium

Gary V. Martin, James H. Caldwell, Janet S. Rasey, Zdenka Grunbaum, Manuel Cerqueira, and Kenneth A. Krohn

Division of Cardiology, Department of Medicine and the Departments of Radiology, Radiation Oncology and Medicine, Seattle V.A. Medical Center and the University of Washington, Seattle, Washington

The 2-nitroimidazole fluoromisonidazole is metabolically trapped in viable hypoxic cells in inverse proportion to PO_2. This attribute suggests that [^{18}F]fluoromisonidazole may be useful for imaging hypoxic tissue using positron emission tomography. To examine this potential, we studied the pharmacokinetics and biodistribution of [^{3}H]fluoromisonidazole in six open chest dogs. In two normal dogs, plasma and urine samples were collected over a 4-hr period following i.v. injection of the drug. In four animals, regional myocardial ischemia was produced 2 hr prior to drug injection by occlusion of the circumflex coronary artery and maintained during the 4-hr sampling period. In all animals, postmortem samples of myocardium and other organs were obtained and tissue, plasma, and urine tritium activity were determined by liquid scintillation counting. In areas of reduced flow, [^{3}H]fluoromisonidazole accumulated in myocardium in inverse proportion to myocardial blood flow measured by microspheres, indicating enhanced binding in hypoxic tissues. Maximum tissue concentrations in ischemic myocardium were two- to three-fold greater than in normal myocardium and plasma. Plasma clearance data indicate the drug is rapidly distributed into the total-body water, clears from the body with a half-life of 275 ± 50 min, and undergoes minimal metabolism by 4 hr. We conclude [^{18}F]fluoromisonidazole may be a suitable agent for radionuclide imaging of hypoxic myocardium.

J Nucl Med 30:194–201, 1989

Mammalian myocardial cells require some minimal oxygen tension to sustain an optimal bioenergetic and contractile state. Oxygen deficiency is a proposed basis for the metabolic and functional abnormalities associated with ischemic heart disease. In the normal heart, local "microhypoxia" may be a key element in the regulatory processes which provide for the close matching of oxygen utilization and myocardial blood flow (*1,2*). Yet there are no clinically useful methods of detecting hypoxic myocardium.

In man, abnormalities in relative regional blood flow provide indirect evidence for myocardial hypoxia. Yet measurement of blood flow alone does not adequately account for other factors which may influence oxygen delivery such as blood oxygen content, tissue extraction, arteriovenous diffusional shunting (*3*), microvascular spacing (*4*), or other factors which may influence the rate of oxygen diffusion in tissue (*5*). Furthermore, cellular PO_2 reflects the balance between oxygen delivery and oxygen utilization and any estimates of myocardial oxygenation based solely on indices of delivery must be considered incomplete.

A unique strategy for the detection of myocardial hypoxia in vivo has evolved from studies of the 2-nitroimidazole misonidazole and its congeners. These compounds are covalently bound in viable hypoxic cells in inverse proportion to PO_2 (*6–8*). Furthermore, binding is enzyme dependent and therefore restricted to viable cells. This relationship has been established most extensively in mammalian tumor cells in vitro, but a recent study of drug binding in an experimental stroke model suggests the relationship may be generalized to nonmalignant tissues (*9*). The incentive to study these

Received Feb. 29, 1988; revision accepted Oct. 11, 1988.

For reprints contact: Gary V. Martin, MD, Dept. of Cardiology (111c), Veterans Administration Medical Center, 1660 South Columbian Way, Seattle, WA 98108.

compounds has been further enhanced by the synthesis of fluorine-18 (^{18}F)fluoromisonidazole (*10*), which may provide a tool for the noninvasive detection of hypoxic but viable tissue using positron emission tomographic (PET) imaging.

The goals of this study were to (a) document enhanced binding of hydrogen-3 (^{3}H) fluoromisonidazole in hypoxic myocardium and (b) determine whether its pharmacokinetics and biodistribution in vivo are compatible with its use as a PET imaging marker for hypoxic myocardium.

METHODS

Hydrogen-3 Fluoromisonidazole

Hydrogen-3 fluoromisonidazole was synthesized in our laboratory by the method described by Grunbaum et al. (*11*). The radiochemical purity as determined by HPLC was > 99%. The specific activities of the two lots of drug used in these studies were 13.78 and 128 μCi/mg, respectively. Hydrogen-3 fluoromisonidazole was stored in a dilute solution in absolute ethanol at 5°C to minimize radiolytic decomposition.

Animals and Surgical Procedures

Six mongrel dogs weighing 20–25 kg were premedicated with intravenous thiamyl sodium (20 mg/kg) and anesthetized with Halothane. Following endotracheal intubation, ventilation was maintained with a Harvard respirator. A minimum arterial oxygen saturation of 97% and pH of 7.40–7.44 was maintained in all animals. The electrocardiogram was continuously monitored.

A left lateral thoracotomy was performed and polyethylene catheters were placed in the descending thoracic aorta and left atrial appendage for blood pressure monitoring and microsphere blood flow measurements. Additional catheters were placed in the left internal jugular and foreleg veins. In two normal animals, a 20-gauge catheter was secured in the coronary sinus. In four others, the circumflex coronary artery was dissected free and a screw clamp occluder placed around the artery. In four animals (two normal, two ischemic), a plastic catheter was advanced retrograde into the bladder which was emptied initially. Hematocrits measured at the end of surgery ranged from 34–42%.

PROTOCOL

In the two dogs without coronary occlusion, [^{3}H]fluoromisonidazole (0.60–0.75 mg/kg) was injected via the left internal jugular vein and arterial and coronary sinus blood samples (0.2 ml/sample) were drawn into pre-heparinized plastic syringes. Samples were taken each 20 sec for the first 4 min following injection and each minute for the first 20 min. Thereafter, samples were obtained every 20 min up to 4 hr postinjection. All samples were promptly centrifuged and the plasma was placed in pre-weighed vials for scintillation counting.

The other four dogs received prophylactic lidocaine consisting of a 2 mg/kg loading dose followed by a continuous infusion of 1 mg/min. One animal received an additional 60 mg for ventricular tachycardia during the experiment. The circumflex coronary artery was occluded for 2 hr prior to drug injection, and the occlusion was maintained for four additional hours while arterial samples were collected as above. In four dogs (two normal, two ischemic), urine samples were collected hourly.

In each animal, regional myocardial blood flow was measured 2 hr post-drug injection by the microsphere technique (*12*). Radiolabeled cobalt-57, 15-micron plastic microspheres (4×10^{6}) were pre-warmed, sonicated for a minimum of 15 min, vortex agitated and immediately injected into the left atrium. A reference blood sample was collected from the aortic catheter at a constant rate of 10 ml/min for 1 min, starting 5 sec before microsphere injection.

TISSUE SAMPLING

Four hours post drug injection, the animals were killed by a lethal bolus of potassium chloride. The heart was rapidly excised and rinsed in iced saline. The left ventricle was dissected free of the remainder of the heart and cut into five transverse rings from base to apex. Any fat or major epicardial vessels were trimmed and the mid left ventricular ring was placed on dry ice to facilitate further sectioning. From the hearts subjected to coronary ligation, rings adjacent to the mid left ventricular ring were immersed in nitro blue tetrazolium (NBT) solution for determination of the infarct areas. The mid left ventricular ring was not stained to avoid color quenching in liquid scintillation counting. This ring was sectioned into 16 radial segments which were further divided into six transmural pieces or a total of 96 locatable pieces of ~ 300 mg in size. The pieces were placed in vials, weighed, and microsphere activity determined by gamma well counting. Regional myocardial blood flow was calculated as $Qm = (Cm \times Qr)/Cr$ where Qm is myocardial blood flow, Cm is counts per minute (cpm) in the tissue samples, Qr is the withdrawal rate of the reference arterial sample and Cr is cpm in the reference sample. Postmortem samples (100–200 mg) of liver, kidney cortex, skeletal muscle, brain, and lung were also obtained.

The tritium activity of all tissue, plasma, and urine samples was determined by liquid scintillation counting. Samples were solubilized in Soluene (Packard, Downers Grove, IL) and counted in Instagel (Packard) scintillation fluid. For each study, the ratio of ^{3}H channel counts to carbon-14 (^{14}C) channel counts in test samples of cold mouse liver mixed with microspheres was determined over a range of sample sizes corresponding with the experimental samples. Even though the samples contained no ^{14}C, dual channel counting allowed determination of the microsphere contribution to total tritium channel counts, which were then subtracted from the total to obtain the activity due to the tritium-labeled drug alone. Each sample was counted to a minimum of 500 counts, therefore the counting variability was small relative to the observed variability between normal and ischemic tissues. Data for tissue and plasma are expressed as dpm/g.

THIN LAYER CHROMATOGRAPHY

Samples of plasma and urine at 15, 30, 60, 120, and 240 min were analyzed by thin layer chromatography (TLC) to

test for the presence of metabolites. Plasma samples were treated (1:2 tissue to solution) with protein precipitating solution (3:1 methanol/acetonitrile) and microfuged for 4 min. The protein pellet was washed and sonicated twice with ethanol. Samples of the serial washes and the residual pellet were taken for liquid scintillation counting. The plasma supernatant was concentrated in a stream of nitrogen to ~ 50% of its original volume and 3 × 5 μl samples were spotted on the TLC plate. Urine was microfuged and 3 × 5 μl samples were also used. Metabolites of biologic samples were identified by running standard solutions of desmethylmisonidazole (DMM) and fluoromisonidazole on each plate.

Two TLC systems were used and replicate biological samples were analyzed using both methods. System 1 used aluminum-backed normal phase plates: silica gel 60 F_{245} (EM 5534) and a mobile phase of chloroform/methanol (93:7). System 2 used $C_{18}RPF_{254}$ plates (Whatman 4803–600) with a mobile phase of methanol/0.5M NaCl (35:65). At the end of the elution, the silica gel plates were cut into 1-cm sections; the reverse phase plates were scraped in 1-cm sections. Samples were counted in a liquid scintillation spectrometer and converted to dpm using a standard quench curve. Standards were visualized under a uv lamp at a wave length of 254 nm.

DATA ANALYSIS

The tritium activity of tissue and plasma samples is expressed as dpm/g. Regional myocardial blood flow values are reported as ml/min/g. To minimize differences in myocardial blood flow between animals, the data for each heart were normalized as decimal fractions of the mean value in nonischemic areas. For hearts subjected to ischemia, the mean normal values for flow were calculated from the central-most 48 samples of the nonischemic region of the ventricle, determined by visual inspection of the location of NBT staining in adjacent myocardial slices. To facilitate comparison of [^{3}H] fluoromisonidazole deposition at different levels of normalized flow, the 96 data points for each experiment were grouped according to the normalized flow value into increments of 0.1 from 0 to 1.5. The mean tritium concentrations of samples within each flow interval were compared to the final plasma tritium concentration. All reported comparisons of ^{3}H activity were made by analysis of variance, with individual differences subjected to the Bonferroni correction.

RESULTS

Administration of [^{3}H]fluoromisonidazole resulted in no detectable changes in heart rate, blood pressure or the electrocardiogram in any of the animals. Figure 1A shows the biphasic plasma clearance of [^{3}H]fluoromisonidazole. The rapid initial distribution phase is followed by a first-order elimination phase with a half-life of 275 ± 50 min. The apparent volume of distribution of the drug was obtained by extrapolating the elimination phase data back to time zero and averaged 0.65 ± 0.21 L/kg, suggesting the drug is distributed into the total-body water space. However, significant binding in one of the tissues not measured would mean that this is an overestimate of the actual volume of distribution. Figure 1B shows that in normal myocardium the drug is highly extracted during first pass and reaches equilibrium within 2 min.

Biodistribution data for various organs is shown in Figure 2. Drug binding in normal myocardium and other organs is similar except in the liver and kidney where mean postmortem tissue concentrations are higher than final plasma concentrations. The explanation for increased liver concentration is unknown. Kidney concentrations were quite variable and this difference did not achieve statistical significance in this small group of animals. Urine concentrations were 50–80 times plasma, indicating this as the primary route of

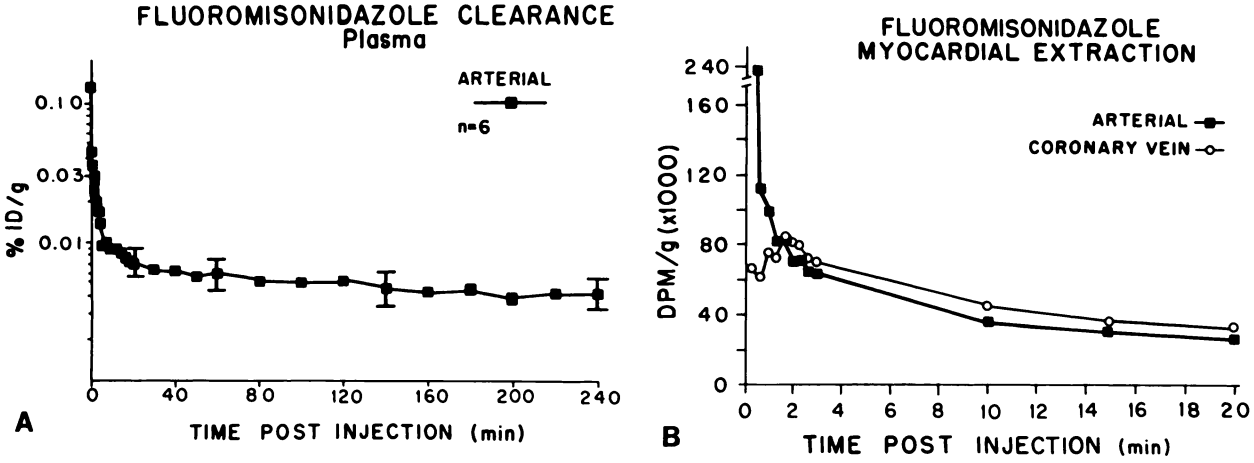

FIGURE 1

A: Plasma clearance [^{3}H]fluoromisonidazole. The data are the mean for all six animals. The rapid distribution phase results from distribution of the drug into the total-body water space (apparent volume of distribution = 0.65 ± 0.21 L/kg). The clearance phase reflects urinary excretion with a clearance $T_{½}$ = 275 ± 50 min. B: Myocardial extraction data were obtained by simultaneous sampling of arterial and coronary sinus blood in dogs without coronary stenosis. Shown are the averaged data from two dogs. Initial myocardial extraction of fluoromisonidazole is ~ 70%.

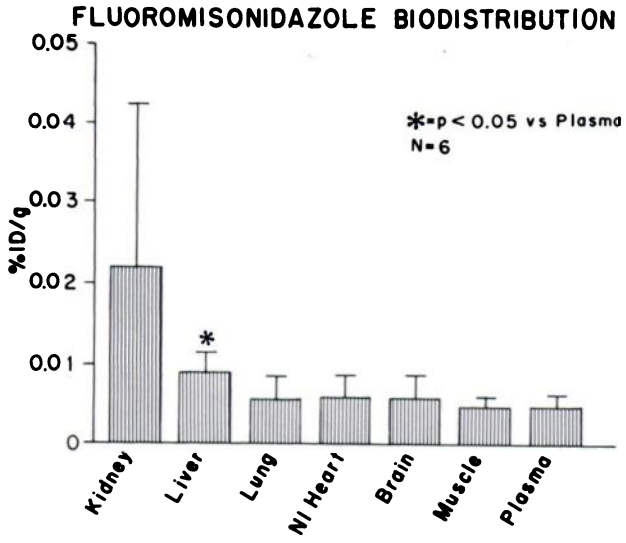

FIGURE 2
Biodistribution of [^{3}H]fluoromisonidazole in normal tissues 4 hr postinjection. The data are the mean for all six animals. Except for kidney and liver, tissue concentrations approximate those of the final plasma concentration.

drug excretion. Elevated concentrations of [^{3}H]fluoromisonidazole in the kidney samples could reflect inclusion of variable amounts of urine in the samples. No differences in urinary concentrations of the drug were present between dogs with (N = 2) and without ischemia (N = 2). We did not measure uptake in fat in this study because previous biodistribution studies in our laboratory have indicated very low retention in fat (~ 20% of that in brain, muscle and other normal tissues). TLC analysis of plasma and urine demonstrated that > 80% of the radioactivity present in urine and virtually 100% of plasma activity at 4 hr was in the parent compound. Importantly, there was no evidence of drug defluorination.

The myocardial blood flow and fluoromisonidazole uptake data for a representative experiment is shown in Figure 3. In nonischemic myocardium, drug deposition is fairly uniform over the threefold range of normal flows. In ischemic areas, drug binding is inversely related to myocardial blood flow. The exception is in areas of absolute lowest flow where final fluoromisonidazole concentrations are equal to but do not exceed those of nonischemic areas.

Figure 4 is a computer-generated "bull's-eye" display of the anatomic location of each of the data points shown in Figure 3. As shown, enhanced fluoromisonidazole uptake occurred in a spatially confluent area of the myocardium corresponding to the area of reduced myocardial blood flow. Areas of low flow not showing increased drug binding are confined to the most subendocardial portion of the ischemic zone.

Grouped data for all six studies are shown in Figure 5. On average, significant increases in tissue concentrations of [^{3}H]fluoromisonidazole were found in areas where blood flow was reduced to half of normal, and highest concentrations were achieved when flow was 20–30% of normal. Drug binding was not enhanced in areas with flow < 10% of normal.

In three of the four dogs subjected to 6 hr of coronary ligation, NBT staining demonstrated a transmural infarction involving 36–48% (by weight) of the left ventricle. In the fourth dog, collateral flow was 30% of normal in the subendocardium and areas of infarction were confined to the most subendocardial layer of the six transmural layers into which the heart was sectioned. The distribution of [^{3}H]fluoromisonidazole deposition relative to myocardial blood flow and areas of infarction are shown in Figure 6. As shown, enhanced drug deposition is not confined to infarcted areas, but includes adjacent areas of only moderately decreased blood flow (38–57% of normal) as well.

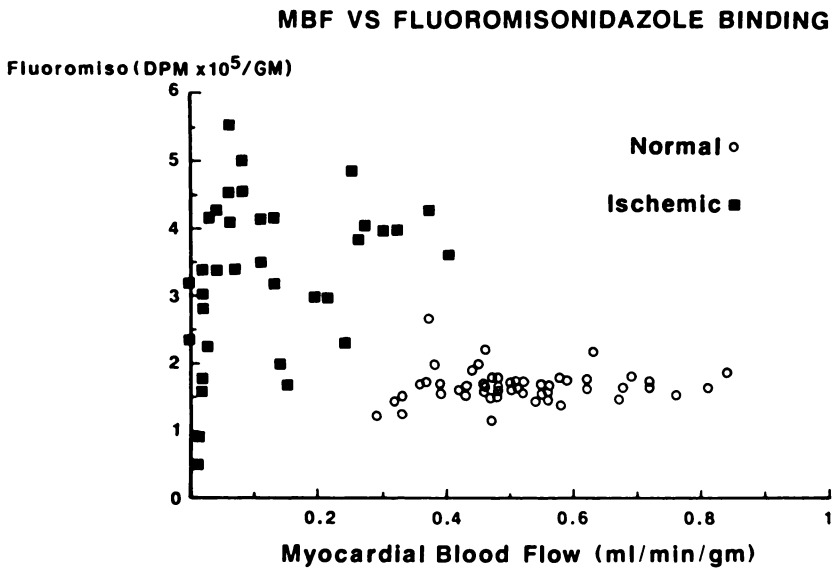

FIGURE 3
Myocardial blood flow and [^{3}H]fluoromisonidazole data from a single animal. The data points represents individual tissue pieces comprising a single mid-left ventricular slice of myocardium.

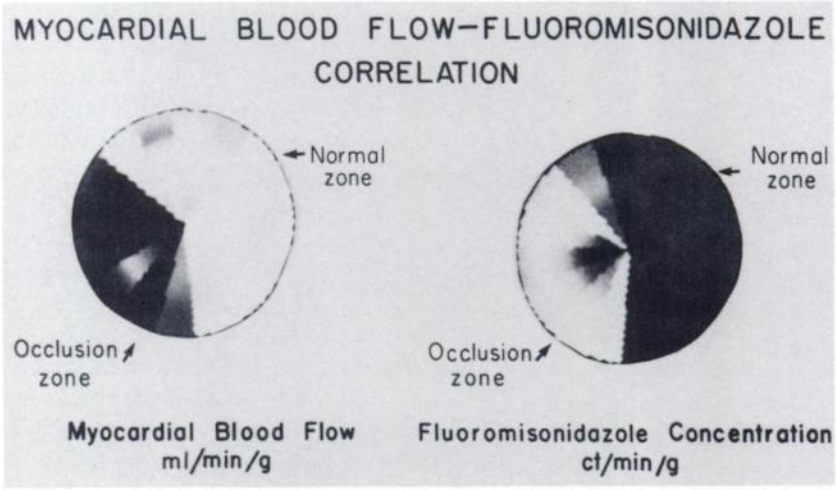

FIGURE 4
Data for microsphere MBF and [^{3}H] fluoromisonidazole binding from a dog subjected to circumflex coronary occlusion. The data are displayed in a computer-generated "bull's-eye" format representing a single transverse slice of left ventricle viewed from the base. Areas of lowest flow (dark) correspond to areas of highest drug binding (light). Lack of enhanced binding in the subendocardial layers of the ischemic zone presumably is because these areas were infarcted at the time of drug injection 2 hr postcoronary occlusion.

DISCUSSION

In simplest terms, myocardial oxygenation is a function of the balance between oxygen delivery and utilization. As oxygen is the most important metabolic substrate of the heart, an externally detectable marker of an imbalance in this relationship would provide important metabolic information. The current study documents enhanced retention of fluoromisonidazole in ischemic myocardium and supports the hypothesis that [^{18}F]fluoromisonidazole may be suitable for imaging hypoxic myocardium using PET.

Numerous investigators have demonstrated the oxygen-dependent binding of misonidazole and fluoromisonidazole in tumor cell monolayers and multicellular spheroids in vitro, and in rodent solid tumors in vivo (*6–8,13,14*). A study by Hoffman et al. (*9*) has provided further evidence that nitroimidazoles may be useful markers of hypoxia in nonmalignant tissues in vivo. In gerbils subjected to unilateral carotid, ligation binding of misonidazole in the cerebral hemisphere ipsilateral to the ligation was significantly increased as compared to the contralateral hemisphere and correlated with stroke severity. Preliminary studies have also indicated increased retention of fluoromisonidazole in heart cells. Shelton et al. noted increased net extraction of the drug in isolated rabbit hearts perfused for 15 min with a hypoxic buffer solution (*15*). Cerqueria et al. measured

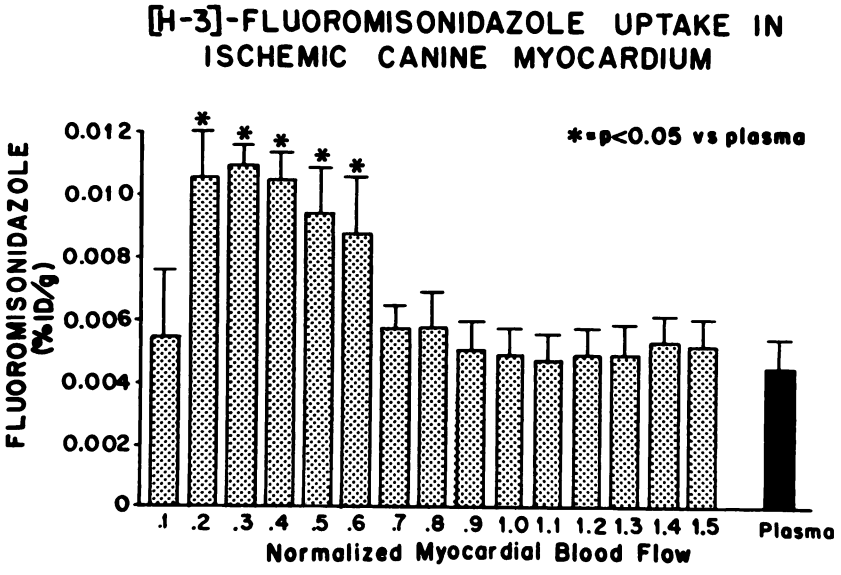

FIGURE 5
Deposition of [^{3}H]fluoromisonidazole as a function of regional myocardial blood flow (measured by microspheres) in dogs subjected to coronary ligation. The data are the mean for all six animals. To minimize differences in blood flow between dogs, the blood flow data (ml/min/g) for each dog were normalized as decimal fractions of the mean blood flow in normal areas of myocardium for that animal before the data were pooled. To compare [^{3}H]fluoromisonidazole deposition in regions with various flows, the data were grouped according to the normalized flow data into intervals of 0.1 from 0. to 1.5. Each bar in the above histogram shows the mean ± s.e.m. [^{3}H]fluoromisonidazole value for all data points falling within the indicated flow interval. The * indicate values which are significantly different from the final plasma concentration. As shown, [^{3}H]fluoromisonidazole deposition is increased by approximately two-fold over plasma in areas where flow is between 10–60% of normal.

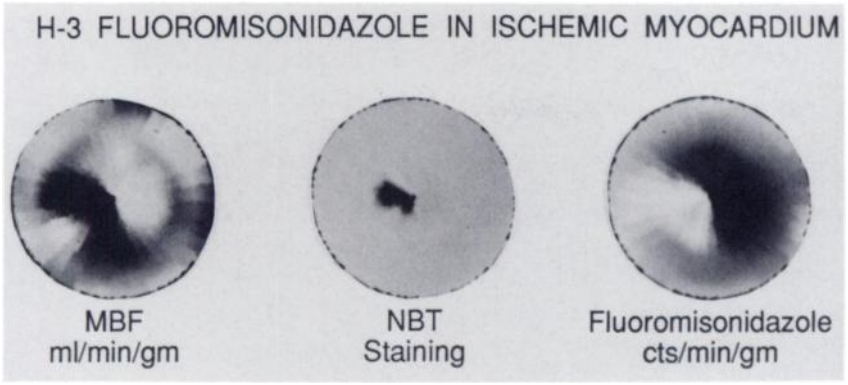

FIGURE 6
The distribution of [^{3}H]fluoromisonidazole relative to myocardial blood flow and NBT staining in a dog with a nontransmural infarction. Enhanced drug deposition (light) occurs outside the zone of infarction (dark) in areas of only moderately decreased flow.

uptake of [^{3}H]fluoromisonidazole in isolated rat myocytes. Under anoxic conditions, binding was increased by greater than tenfold by 180 min (*16*).

Although unproven, the favored mechanism of drug binding involves reduction of the nitro group to a reactive anion radical. The nitro anion radical (the one electron product) is a metabolite which, when reduced by a second electron, is committed to bind to intracellular molecules. In the presence of oxygen, the favored reaction is reformation of the less reactive parent compound in a futile cycle (Fig. 7). The parent compound has a partition coefficient of 0.42 and is therefore quite freely diffusible, is not bound and clears from the tissues as a function of time. Dead tissue fails to bind these compounds because the enzymic capacity is lacking.

The inverse relationship between regional fluoromisonidazole deposition and myocardial blood flow provides evidence for enhanced binding of the drug in hypoxic myocardium. Statistically significant increases in drug binding were present in myocardium where flow was reduced to 10–60% of normal. The ratios of drug binding in these areas to that in nonischemic tissue was 1.8–2.4, a difference large enough to detect by external nuclear imaging. Importantly, this difference was present within a time frame (4 hr) compatible with the nuclear half-life of ^{18}F.

It is unlikely that enhanced fluoromisonidazole retention resulted from delayed washout of the drug from ischemic myocardium. Final tissue concentrations of the drug were nearly twice final plasma concentrations in areas of only modest flow reductions (50–60% of normal). It seems unlikely that washout of this very diffusible drug would be substantially inhibited by such a modest flow reduction at 4 hr postinjection.

A potential limitation of any "positive" marker of tissue hypoxia is that low flow may restrict access of the drug to the target areas. This study demonstrates that tissue deposition of fluoromisonidazole is adequate even in areas of quite low flow (10–20% of normal). In the lowest flow (< 10%) areas, final tissue concentrations were equal to plasma, suggesting adequate drug delivery. Figure 4 shows that regions of low flow and nonenhanced fluoromisonidazole uptake were confined to the most central subendocardial portion of the ischemic zone. Since these areas were likely to have been infarcted at the time of administration 2 hr postcoronary occlusion, we hypothesize that enhanced drug retention did not occur because the tissue was already necrotic and, therefore, lacked the enzymatic capacity to bind the drug. This explanation is certainly compatible with in vitro observations that binding does not occur in dead cells.

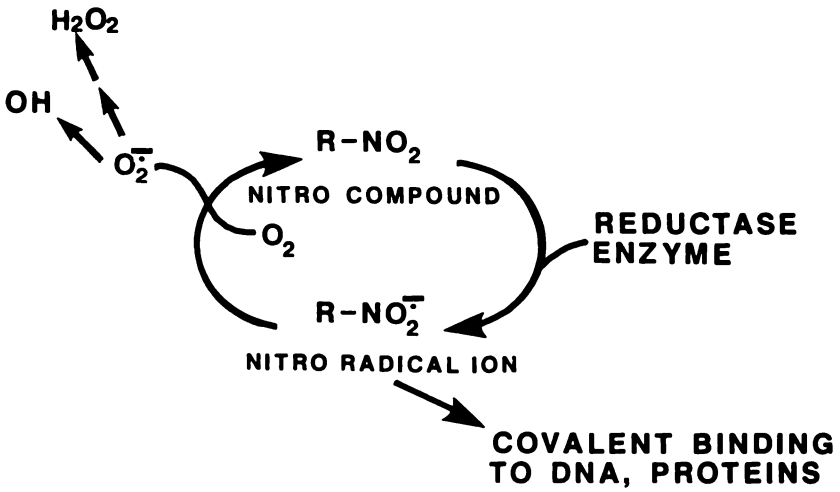

FIGURE 7
The mechanism of intracellular binding of nitroimidazoles. Reductase enzymes convert the parent compound to a nitro radical anion which is bound intracellulary. In the presence of oxygen, the parent compound is generated.

While myocardial oxygen tensions were not measured in this study, the NBT staining results provide solid evidence that the areas of enhanced drug binding were severely hypoxic during drug exposure. Data from the one animal in which the infarct was nontransmural (Fig. 6) also provides evidence that less severe degrees of hypoxia, not resulting in infarction, also promote drug binding.

In normal myocardium, the drug is highly extracted initially and arterial and coronary sinus plasma concentrations rapidly reach equilibrium. Initial extraction in ischemic myocardium was not measured in this study. In each study the final concentration of drug in normal tissues approximated that of plasma. These observations are consistent with the hypothesis that the drug distributes nonselectively into body tissues, and that tissue concentrations remain in equilibrium with plasma in the absence of hypoxia. In hypoxic tissues, an intracellular reaction acts to retain and concentrate the drug in excess of plasma concentration.

A limitation of this study is that regional myocardial blood flow was measured at a single time during the experiment. The reason for this is that virtually all of the commercially available, commonly used radiolabeled microspheres either have beta emissions as part of their decay or contain low level contaminants with energy peaks in the same range as tritium, thus complicating determination of tritium activity. An important assumption, therefore, is that this "snapshot" estimate of flow adequately reflects the distribution of average flows for the entire experimental period. Data from other sources suggests that this is the case. While there are temporal heterogeneities in regional flows, these are relatively small compared to spatial heterogeneities (*17*). Thus, areas of relatively higher or lower flows tend to remain so over time. Previous studies have also shown that total collateral flow to infarct zones is relatively stable between 2 and 6 hr postocclusion (*18,19*).

In summary, these studies provide evidence for enhanced binding of the hypoxic cell marker fluoromisonidazole in ischemic myocardium. The available data from this and other studies implicate cellular hypoxia as the principal reason for increased tissue retention. The significance of these results lies in the possibility that [^{18}F]fluoromisonidazole can be used to image hypoxic myocardium in man with PET. Potentially, this agent could provide information quite unique from and complementary to that offered by currently used cardiac imaging agents. Myocardial hypoxia is a metabolic state not specifically targeted by flow markers such as thallium, whereas infarct-avid agents such as Tc-pyrophosphate or indium-labeled antimyosin antibodies require cell necrosis. In theory, fluoromisonidazole could be used to identify hypoxic but viable myocardium.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Marilou Gronka, Lay Chin, and Sara Magee in the performance of the experiments and of Sylvia Duncan and James Kousbaugh in the preparation of the manuscript.

This work was supported by the Veterans Administration General Medical Research Service, the American Heart Association-Washington Affiliate, the NIH RO1 CA34570 and PO1 HL38736.

REFERENCES

1. Olsson RA, Bunger R. Metabolic control of coronary blood flow. *Prog Cardiovasc Dis* 1987; 29:369–387.
2. Schubert RW, Whalen WJ, Nair P. Myocardial PO_2 distribution: relationship to coronary autoregulation. *Am J Physiol* 1978; 234:H361–H370.
3. Roth AC, Feigl EO. Diffusional shunting in the canine myocardium. *Circ Res* 1981; 48:470–480.
4. Turek Z, Rakusan K. Lognormal distribution of intercapillary distance in normal and hypertrophic rat heart as estimated by the method of concentric circles: its effect on tissue oxygenation. *Pflugers Arch* 1981; 391:17–21.
5. Honig CR, Gayeski TEJ, Federspiel W, Clark A, Clark P. Muscle O_2 gradients from hemoglobin to cytochrome: new concepts, new complexities. *Adv Exp Med Biol* 1984; 169:23–38.
6. Chapman JD, Baer K, Lee J. Characteristics of the metabolism-induced binding of misonidazole to hypoxic mammalian cells. *Cancer Res* 1983; 43:1523–1528.
7. Rasey JS, Krohn KA, Grunbaum Z, Conroy PJ, Bauer K, Sutherland RM. Further characterization of 4-bromomisonidazole as a potential detector of hypoxic cells. *Radiat Res* 1985; 102:76–85.
8. Rasey JS, Grunbaum Z, Magee S, Nelson NJ, Olive PL, Durand RE, Krohn KA. Characterization of radiolabeled fluoromisonidazole as a probe for hypoxic cells. *Radiat Res* 1987; 111:292–297.
9. Hoffman JM, Rasey JS, Spence A, Shaw D, Krohn K. Binding of the hypoxia tracer [3H]misonidazole in cerebral ischemia. *Stroke* 1987; 18:168–176.
10. Jerabek PA, Patrick TB, Kilbourn MR, Dischino DD, Welch MJ. Synthesis and biodistribution of 18F-labeled fluoromisonidazoles: potential in vivo markers of hypoxic tissue. *Appl Radiat Isot* 1986; 37:599–605.
11. Grunbaum Z, Freauff SJ, Krohn KA, Wilbur DS, Magee S, Rasey JS. Synthesis and characterization and congeners of misonidazole for imaging hypoxia. *J Nucl Med* 1987; 28:68–75.
12. Heymann MA, Payne BD, Hoffman JIE, Rudolph AN. Blood flow measurements with radionuclide labeled particles. *Prog Cardiovasc Dis* 1977; 20:55–79.
13. Chapman JD, Franko AJ, Sharplin J. A marker for hypoxic cells in tumours with potential clinical applicability. *Br J Cancer* 1981; 43:546–550.
14. Koch CJ, Stobbe CC, Baer KA. Metabolism induced binding of C-14 misonidazole to hypoxic cells: kinetic dependence on oxygen concentration and misonidazole concentration. *Int J Radiat Oncol Biol Phys* 1984; 10:1327–1332.
15. Shelton ME, Welch MJ, Dence CS, Hwang DR, Berg-

mann SR. [F-18]fluoromisonidazole: a potential marker of salvageable myocardium. *J Nucl Med* 1988; 29:807.
16. Cerqueria 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* 1988; 29:807.
17. King RB, Bassingthwaighte JB, Hales JRS, Rowell LB. Stability of heterogeneity of myocardial blood flow in normal awake baboons. *Circ Res* 1985; 57:285–295.
18. Rivas F, Cobb FR, Bache RJ, Greenfield JC. Relationship between blood flow to ischemic regions and extent of myocardial infarction. *Circ Res* 1976; 38:439–447.
19. Bishop SP, White FC, Bloor CM. Regional myocardial blood flow during acute myocardial infarction in the conscious dog. *Circ Res* 38: 429–438.