Characterization of [^{18}F]Fluoroetanidazole, a New Radiopharmaceutical for Detecting Tumor Hypoxia

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Fluorinated derivatives of etanidazole are being explored as probes for tumor hypoxia. Our research group has synthesized [^{18}F]fluoroetanidazole (FETA) and now reports the oxygen dependency of binding to cells in vitro, the biodistribution of the tracer in tumor-bearing mice and the analysis of metabolites in their plasma and urine. **Methods:** Four cultured rodent cell lines (V79, 36B10, EMT6 and RIF1) were incubated with [^{18}F]FETA for various times under graded O_2 concentrations. We also compared the biodistributions of [^{18}F]FETA and [^{18}F]fluoromisonidazole (FMISO) at 2 and 4 h postinjection in C3H mice bearing KHTn tumors (130–430 mg). Reverse-phase high-performance liquid chromatography was used to distinguish metabolites from parent drugs in urine and plasma of mice injected with [^{18}F]FETA or [^{18}F]FMISO. **Results:** In cells labeled in vitro, O_2 levels of 600–1300 ppm inhibited binding by 50% relative to uptake under anoxic conditions (<10 ppm). These inhibitory values are not statistically different from those reported for [^{18}F]FMISO in the same cell lines (700–1500 ppm). In the biodistribution studies, uptake in heart, intestine, kidney and tumor was similar for both tracers 4 h after injection, whereas retention of [^{18}F]FETA in liver and lung was significantly lower. Less uptake of [^{18}F]FETA in liver suggests that this nitroimidazole is metabolized less than [^{18}F]FMISO. The brain-to-blood ratios indicate that [^{18}F]FETA readily crosses the blood-brain barrier. High-performance liquid chromatography of urine demonstrated that 10% of [^{18}F]FETA-derived activity was in metabolites at 2 h postinjection, with 15% in metabolites by 4 h; comparable values for [^{18}F]FMISO were 36% and 57%, respectively. **Conclusion:** We conclude from these data that [^{18}F]FETA holds promise as a new hypoxia tracer in patients, having oxygen dependency of binding similar to [^{18}F]FMISO in vitro and displaying less retention in liver and fewer metabolites in vivo.

Key Words: hypoxia; [^{18}F]fluoroetanidazole; tumor imaging; [^{18}F]fluoromisonidazole

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Oncologists recognize that hypoxia in human tumors influences response to treatment and also may play a role in tumor aggressiveness and propensity to metastasize. Radiation therapists have long recognized the association between reduced tumor oxygenation and poor response to radiation treatment (*1,2*), and these early observations have been confirmed and extended by modern studies. Low levels of tumor oxygenation, measured with polarographic oxygen electrodes and typically defined as the proportion of oxygen partial pressures at or below the range of 2.5–10 mm Hg, show a strong correlation with poor outcomes in head and neck cancer and cervical carcinoma treated with radiation (*3*).

More recently, tumor hypoxia has been implicated in more aggressive tumor growth, greater propensity to metastasize and resistance to some chemotherapeutic agents. Most of these studies have used rodent and cell culture models, and some recent reports with human tumors have confirmed the laboratory studies. Höckel et al. (*3*) reported that, in patients with cervical cancer treated with surgery, those with hypoxic tumors (defined as having a median $Po_2 < 10$ mm Hg) were more likely to fail by local tumor extension or spread to regional lymph nodes. Brizel et al. (*4*) reported that patients with soft-tissue sarcomas with a median $Po_2 < 10$ mm Hg were more likely to fail with distant metastases to the lung compared with patients with well-oxygenated tumors. These results suggest that hypoxia may be important in many aspects of tumor biology.

The elegant and informative studies of tumor hypoxia with oxygen electrodes have been limited largely to carcinomas of the cervix or head and neck and soft-tissue sarcomas in the extremities because they are accessible for invasive measurement. This limitation re-emphasizes the importance of developing and characterizing imaging agents that detect and accurately quantify hypoxia without interference from nonspecific binding in well-oxygenated tissues. The nitroimidazole analog [^{18}F]fluoromisonidazole (FMISO; 1H-1-(3[^{18}F]fluoro-2-hydroxypropyl)-2-nitroimidazole) was developed for PET imaging of hypoxia (*5–7*) and now is used in several medical centers worldwide (*8–10*). This lead radiopharmaceutical has proven very useful in assessing hypoxia in human tumors. However, we seek new agents that use the same principle of metabolic trapping (covalent binding of nitroreduction products) but that may be more strictly oxygen dependent across different cell types or metabolized less readily in vivo. Our research group recently reported the

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synthesis of [^{18}F]fluoroetanidazole (FETA; N-(2-fluoroethyl)-2-(2-nitroimidazole-1-yl)-acetamide), a monofluorinated congener of etanidazole with a fluorine on the alkyl side chain (*11*). This analog was developed because other congeners of etanidazole, typified by the pentafluorinated molecule EF5 (N-(2,2,3,3,3-pentafluoropropyl)-2-(2-nitroimidazole-1-yl)-acetamide), showed promise for detecting tumor hypoxia (*12*). EF5 demonstrates less variation in oxygen dependency of binding in different cell lines (approximately two to three times the range of K values) compared with the prototype molecule misonidazole (*13,14*).We report herein the oxygen dependency of the binding of [^{18}F]FETA to tumor cells in vitro, the biodistribution of the radiopharmaceutical in tumor-bearing mice and the analysis of the metabolites appearing in mouse blood and urine.

MATERIALS AND METHODS

Synthesis of [^{18}F]FETA and [^{18}F]FMISO

The methods for synthesis of [^{18}F]FETA were reported by Tewson (*11*). [^{18}F]FMISO was synthesized as described by Grierson et al. (*7*).

Cell Lines and Culture Conditions

The in vitro characterization of [^{18}F]FETA was performed using four transformed cell lines, all of which were examined during exponential growth phase. Mouse mammary sarcoma (EMT6) and fibrosarcoma (RIF1) lines were maintained in DMEM/F-12 medium (Life Technologies, Gaithersburg, MD). A Chinese hamster lung fibroblast line (V79) grown in minimal essential medium with Earle's salts and a rat glioma line (36B10) grown in Waymouth's MB 752/1 (1 × NEAA, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate) also were examined. All media contained 10% fetal bovine serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin. Incubation of all lines was at 37°C with 5% CO_2.

Hypoxia Incubation, Drug Uptake and Counting

To produce conditions of severe hypoxia or specific O_2 concentrations, exponentially growing cells in 60 mm Permanox plates (Nunc, Inc., Naperville, IL) were incubated in medium containing [^{18}F]FETA at 11–36 μCi/mL, 1 mL/plate. Plates were then placed in aluminum gassing chambers that were attached to a manifold with a vacuum pump to allow evacuation of the chambers, followed by an influx of gas at the desired O_2 concentration, as described by Koch (*15*). Seven such gassing cycles were performed in a 30-min period, followed by incubation at 37°C in the sealed chamber for up to 3 h (*15,16*). All gas mixtures contained 5% CO_2. After incubation, plates were rinsed twice with D-PBS to remove residual exogenous compound, cells were enzymatically disaggregated with 0.05% trypsin/0.02% ethylenediaminetetraacetic acid and cells per milliliter were determined with a Coulter ZM electronic particle counter (Coulter Electronics, Inc., Hialeah, FL). Gamma activity of the resulting cell suspension was then measured in a Cobra II gamma counter (Packard Instrument Co., Meriden, CT). Counts were decay-corrected to the time of synthesis.

Mouse Tumor Transplant and Growth

To assess the in vivo biodistribution and metabolism of [^{18}F]FETA, subcutaneous tumors were transplanted medially on the backs of 6-wk-old male C3H/HeJ mice (Jackson Laboratory, Bar Harbor, ME) through injection of 2 × 105 exponentially growing KHTn mouse sarcoma cells. Experiments generally were performed 12 d after injection when tumors had grown to 150–300 mg.

Biodistribution Protocol

Biodistribution of [^{18}F]FETA and [^{18}F]FMISO was examined after intraperitoneal injection of the relevant tracer in saline solution, 300 μCi/mL, 10 μL/g body mass. Two or 4 h after injection, the mice were killed, and organ (brain, jejunum, heart, kidney, liver, thigh muscle, lung) and tumor samples were excised and weighed. Samples (100–200 μL) of urine and cardiac or brachial blood were taken at the same time. Radioactivity was then counted in a Cobra II gamma counter, and data were decay-corrected to time of synthesis and normalized to percentage injected dose per gram (%ID/g) of tissue.

High-Performance Liquid Chromatography Analysis of Blood and Urine from Mice

Urine and plasma samples were analyzed by high-performance liquid chromatography (HPLC) to determine the quantity of labeled metabolites at various time points after injection. Plasma samples were treated with an equal volume of methanol, the proteins precipitated by centrifugation at 1400 rpm in an Eppendorf microcentrifuge and the supernatant pipetted off. The supernatant from the methanol precipitation was passed through a 0.45-μm nylon Acrodisc filter (Gelman, Ann Arbor, MI) to remove remaining debris. HPLC was then performed on a 50-μL sample of urine or deproteinized plasma, injected into a 5-μm C_{18} column (Prodigy ODS 3; Phenomenex, Torrance, CA) running at 2 mL/ min, initially with pure water. Then after 10 min, a gradient was started that reached 100% methanol at 13 min and continued at 100% methanol for an additional 7 min. The column eluant was passed through a radioactivity detector, and in addition, fractions were collected at 1-min intervals and counted in a gamma counter (Packard, Downer's Grove, IL). An additional 50-μL sample of the supernatant was counted for radioactivity at the same time to ensure that there was complete recovery from the column. The ultaviolet absorption at 254 nm was measured as a monitor of system performance but did not provide useful analytical data because of the high specific activity of the ^{18}F label.

RESULTS

The time course of binding of [^{18}F]FETA by oxygenated and severely hypoxic cells is shown in Figure 1. For two of the four cell lines (V79 hamster lung fibroblasts and 36B10 rat glioma cells), binding appears to be linear as a function of time up to 3 h. For the other two cell lines, EMT6 mouse sarcoma and RIF1 mouse fibrosarcoma cells, binding departs slightly from linearity between 1.5 and 3 h. Cell size was measured for all four cell lines, allowing binding at 3 h to be expressed relative to the concentration of ^{18}F activity in the medium. Concentration above activity in the medium was highest for 36B10 glioma cells and lowest for EMT6 sarcoma cells. The concentration ratios for the four cell lines were EMT6: 18.2 ± 4.8-fold concentration above medium levels (n = 5 experiments), RIF1: 28.2 ± 5.6-fold

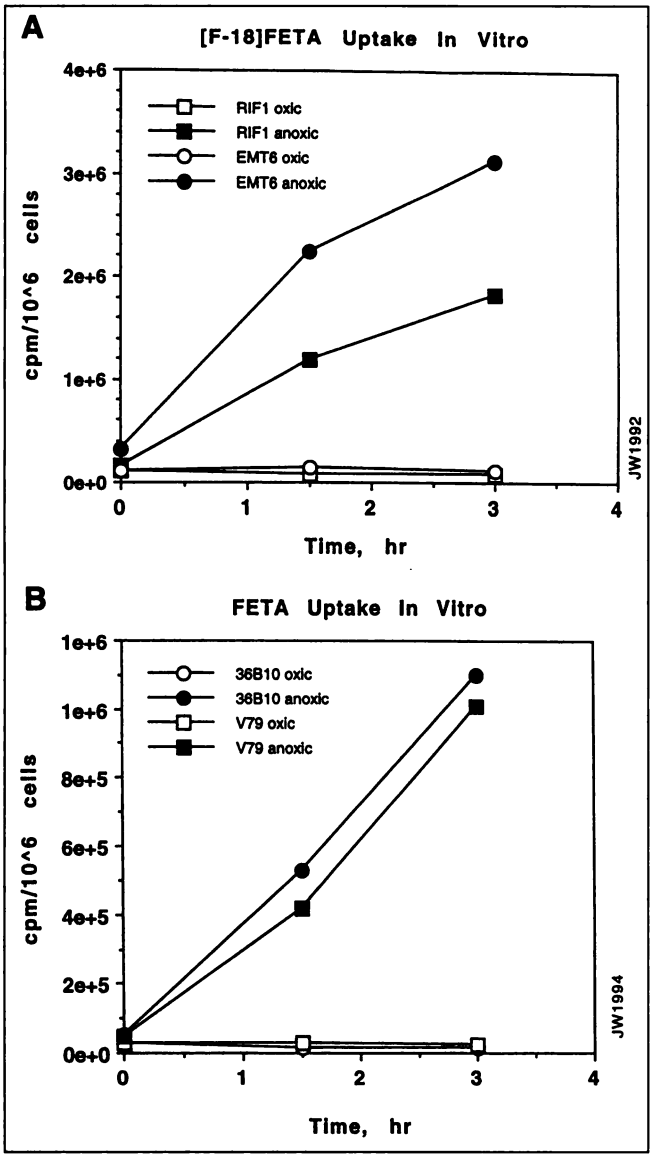

FIGURE 1. Time course uptake of [^{18}F]FETA in cells in vitro incubated with drug in contact with 95% air–5% CO_2 (oxic conditions) or 95% N_2–5% CO_2 (anoxic conditions). (A) EMT6 and RIF1 mouse sarcoma cells. (B) 36B10 rat glioma cells and V79 Chinese hamster lung fibroblasts.

concentration (n = 5), 36B10: 58.2 ± 8.7-fold concentration (n = 4) and V79: 38.8 ± 9.1-fold concentration (n = 4).

The binding of [^{18}F]FETA as a function of O_2 concentration also was measured for the same four cell lines after a 3-h incubation. Binding under near anoxic conditions (10-ppm O_2) was normalized to 100%, and binding at higher O_2 concentrations was related to that value. The O_2 concentration needed to inhibit binding by 50% was determined by linear interpolation, averaged from three separate experiments for each cell line. The average value for each cell line lies between 600- and 1300-ppm O_2 (Table 1). Representative binding curves for 36B10 and RIF1 cells are shown in Figure 2. Retention of [^{18}F]FETA decreases rapidly as O_2 levels increase from 10 to 5,000 ppm (0.5%), with only

TABLE 1
Oxygen Levels that Inhibit [^{18}F]FETA Binding by 50%

Cell type	Oxygen level, ppm to inhibit binding by 50%
EMT6	1010 ± 146
RIF1	1055 ± 593
36B10	1291 ± 840
V79	588 ± 195

FETA = fluoroetanidazole.

modest further decline between 5,000 and 210,000 ppm (air).

The biodistribution of [^{18}F]FETA in C3H mice bearing KHTn tumors was compared with the distribution of [^{18}F]FMISO. Figure 3 shows that the blood clearance for both tracers is remarkably similar, except that [^{18}F]FETA activity peaks slightly sooner after intraperitoneal injection than does [^{18}F]FMISO activity. This could be an artifact of intraperitoneal injection and might not be seen if tracer were injected intravenously. Table 2 summarizes the tissue-to-blood ratios for both tracers for mice killed 2 or 4 h after tracer injection and identifies the tissues and postinjection times when uptake of the two tracers differs significantly ($P < 0.05$). The uptake of the two radiopharmaceuticals into KHTn tumors at 4 h postinjection is similar, but in contrast, their distribution to certain normal tissues differs markedly. The principal difference between the two tracers is that less [^{18}F]FETA is retained by liver and lung than is the case for [^{18}F]FMISO. The lung-to-blood ratio for [^{18}F]FETA is not significantly different from 1.0 at either 2 or 4 h after tracer injection, whereas for [^{18}F]FMISO the lung-to-blood ratio

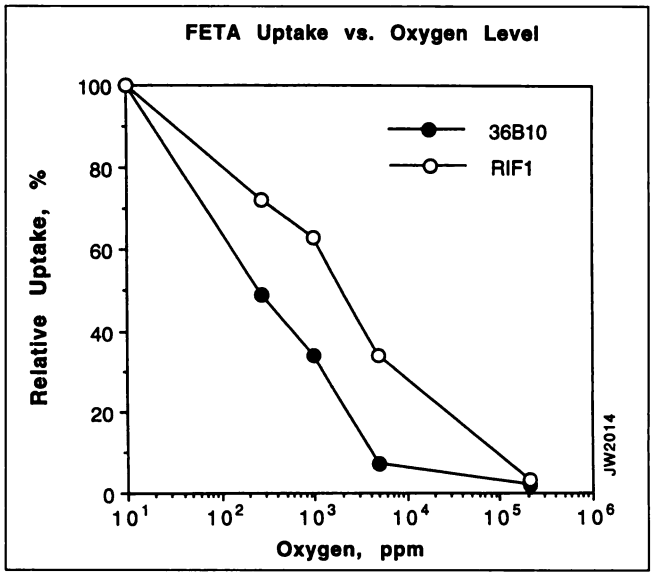

FIGURE 2. Oxygen dependency of binding of [^{18}F]FETA in 36B10 and RIF1 cells. Cells were incubated for 3 h while in contact with variable concentrations of oxygen; balance of gas mixture was CO_2 (5%) and N_2.

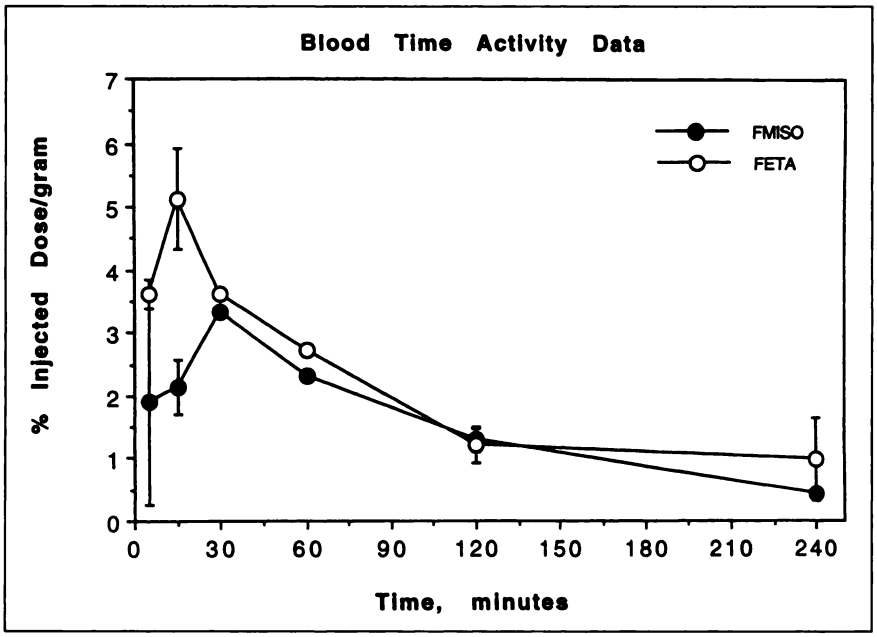

FIGURE 3. Blood time-activity curves for [^{18}F]FMISO and [^{18}F]FETA in C3H/HeJ male mice. Data are expressed as percentage injected dose per gram body weight (%ID/g) for total radioactivity remaining in blood.

approaches 2.0 by 4 h. Although uptake of [^{18}F]FETA in the liver at 4 h is high (6.40 ± 1.64 tissue-to-blood ratio), it is only half the value for [^{18}F]FMISO (12.29 ± 4.37). These two uptake values are highly significantly different (P = 0.006). For muscle, heart and brain, the tissue-to-blood ratios for [^{18}F]FETA are all close to 1.0 at 4 h, an expected result for well-oxygenated normal tissue not involved in metabolism or excretion of nitroimidazoles.

We also performed HPLC analysis of urine from mice injected with [^{18}F]FETA or [^{18}F]FMISO, to determine what proportion of the activity was in unaltered parent drugs or metabolites. Urine samples were taken at 2 or 4 h after injection; the amount of urine voided between injection and sampling was not recorded. Plasma samples taken 2 h after tracer injection also were analyzed. [^{18}F]FETA was metabolized less readily than [^{18}F]FMISO, as shown by a smaller proportion of [^{18}F]FETA appearing as metabolites at both 2 and 4 h (Table 3). The elution profiles for 2-h urine samples, as monitored with the in-line radioactivity detector, are shown in Figure 4. [^{18}F]FETA (Fig. 4A) eluted from the C_{18} column with a peak at 6.7 min. All detectable metabolites were more polar and eluted at earlier times. Unaltered [^{18}F]FMISO (Fig. 4B) eluted at 12.6 min and most metabolites, as was the case with [^{18}F]FETA, were more polar. A less polar metabolite eluted at 13.2 min. A similar [^{18}F]FMISO metabolite also has been seen in human urine (data not shown). At 2 h, 10% of [^{18}F]FETA-derived radioactivity eluted as metabolites, whereas 36% of [^{18}F]FMISO activity appeared as metabolites. By 4 h, these values increased to

TABLE 2
Biodistribution of FETA and FMISO in C3H Mice with KHTn Tumors (Tissue-to-Blood Ratios)*

Tissue	[^{18}F]FETA† 2 h	[^{18}F]FMISO‡ 2 h	P§	[^{18}F]FETA‖ 4 h	[^{18}F]FMISO¶ 4 h	P
Brain	0.86 ± 0.15	0.88 ± 0.11	0.744	1.07 ± 0.31	0.63 ± 0.07	0.002
Gut	2.41 ± 0.97	3.65 ± 1.92	0.179	3.45 ± 1.11	5.40 ± 4.09	0.222
Heart	1.00 ± 0.15	1.04 ± 0.07	0.665	1.08 ± 0.15	0.98 ± 0.09	0.053
Kidney	4.56 ± 2.67	4.56 ± 1.28	0.994	7.20 ± 3.62	6.35 ± 5.18	0.692
Liver	3.28 ± 0.94	6.67 ± 0.95	0.0004	6.40 ± 1.64	12.29 ± 4.37	0.006
Lung	1.08 ± 0.17	1.65 ± 0.25	0.001	1.15 ± 0.12	1.96 ± 0.24	0.00003
Muscle	1.05 ± 0.31	0.93 ± 0.16	0.239	1.03 ± 0.1	0.84 ± 0.17	0.035
KHTn tumor	2.20 ± 0.77	1.40 ± 0.25	0.001	3.84 ± 1.51	3.30 ± 2.00	0.588

*All values are mean ± SD.
†n = 17 for all tissues except heart (n = 16).
‡n = 6 for all tissues.
§P values (2-tailed) were determined using Student t test.
‖n = 13 for all tissues except lung and tumor (n = 6) and muscle (n = 12).
¶n = 8 for all tissues.
FETA = fluoroetanidazole; FMISO = fluoromisonidazole.

TABLE 3
Metabolism of [^{18}F]FMISO and [^{18}F]FETA in Mouse Plasma and Urine

Sample	[^{18}F]FETA (% in metabolites)	[^{18}F]FMISO (% in metabolites)
2 h plasma	31.8	50.0
2 h urine	10.3	36.2
4 h urine	14.8	57.3

FMISO = fluoromisonidazole; FETA = fluoroetanidazole.

15% and 57%, respectively. These comparisons are summarized for both urine and plasma in Table 3.

DISCUSSION

The two radiopharmaceuticals, [^{18}F]FMISO and [^{18}F]FETA, demonstrate similar oxygen dependency of binding in cultured cells. The level of oxygen inhibiting binding by 50% (K value) ranges from 720 to 1550 ppm for [^{18}F]FMISO for the V79, RIF1, EMT6 and 36B10 cell lines (*16*, unpublished data) and from 590 to 1300 ppm for [^{18}F]FETA in the same four cell types (Table 1). The mean K values were 1131 ± 253 ppm (mean ± SEM) for [^{18}F]FETA and 1292 ± 208 ppm for [^{18}F]FMISO. These mean values are not significantly different (P = 0.62, Student t test).

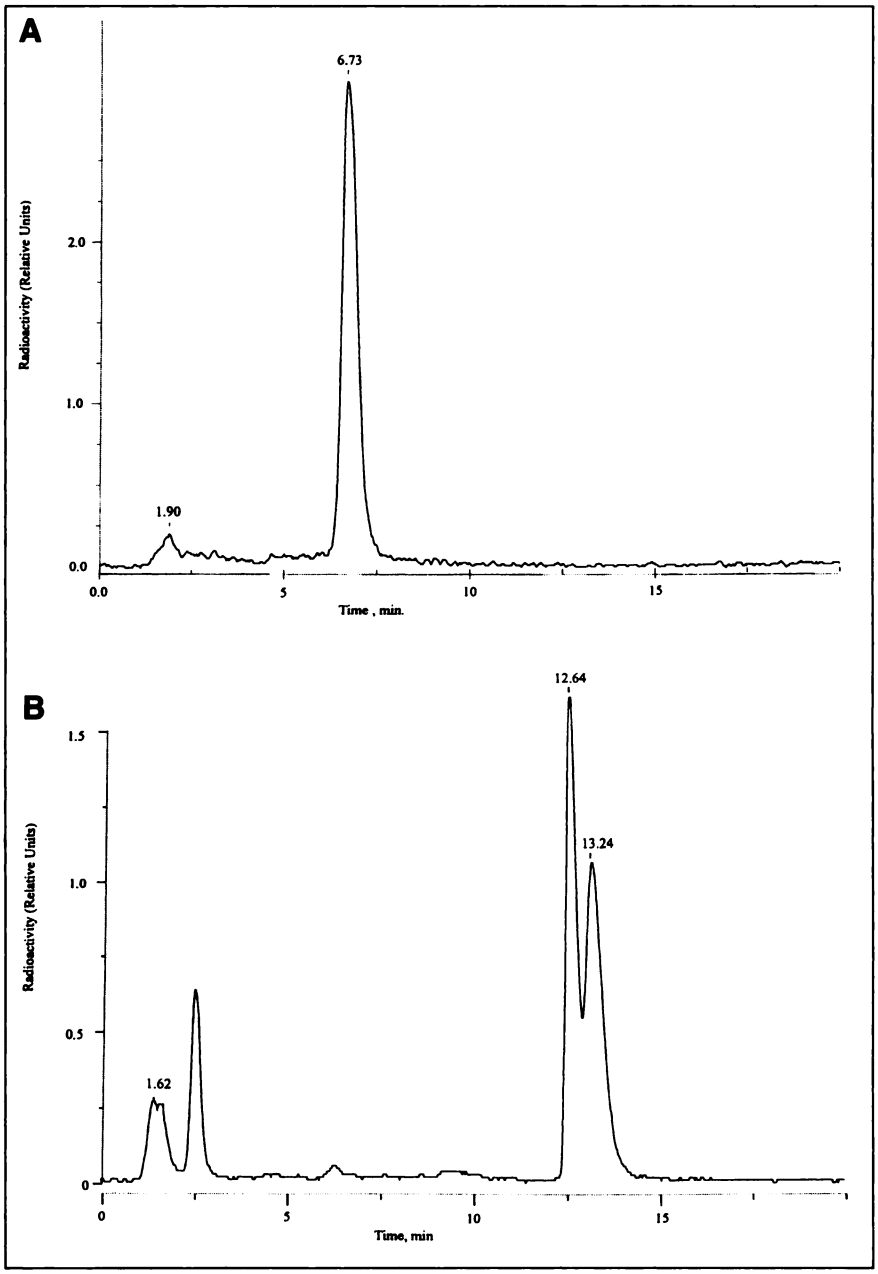

FIGURE 4. HPLC analysis of metabolites in urine of mice injected 2 h earlier with [^{18}F]FETA (A) or [^{18}F]FMISO (B).

These mean values are close to the level of hypoxia that provides maximal protection against radiation, and a radiopharmaceutical that binds at such low levels of O_2 reports hypoxia that is relevant to treatment of cancer with radiotherapy. The similarity of oxygen dependency for the two tracers is predictable. Binding as a function of O_2 level should be directly related to the electron affinity of the NO_2 group in the molecule, which determines how easily it will be reduced. FMISO and the nonfluorinated nitroimidazole from which [^{18}F]FETA is derived (etanidazole) have nearly identical electron affinities (E17 values): −383 mV for [^{18}F]FMISO and −388 mV for etanidazole (*17,18*), and so the similar K values for [^{18}F]FETA and [^{18}F]FMISO would be expected. Studies of another etanidazole derivative, the pentafluorinated EF5 (*12,13*), have characterized this nitroimidazole as a probe for hypoxic cells. EF5 adducts formed in hypoxic cells have been quantified using a ^{14}C-labeled compound as well as fluorescent tagged antibodies to the adducts, detected with immunohistochemistry or flow cytometry. These studies revealed an oxygen K value of 1000 ppm for V79 WRNE cells and approximately 2200 ppm for 9L rat glioma cells, two cell lines that showed wider variation of K values for misonidazole (*13,14*). The two-fold range in mean K values for [^{18}F]FETA and [^{18}F]FMISO with different cell types reported here as well as the absolute values (a mean of 1131 ppm for [^{18}F]FETA and 1292 ppm for [^{18}F]FMISO) are similar to data reported for EF5 (*13*).

In the in vivo biodistribution studies, [^{18}F]FETA and [^{18}F]FMISO were expected to show similar retention by hypoxic tumors on the basis of the similar K values previously reported. The uptake of the two tracers into KHTn tumors 4 h after injection was not significantly different: 3.84 ± 1.51 tumor-to-blood ratio for [^{18}F]FETA and 3.30 ± 2.00 for [^{18}F]FMISO (P = 0.588) in tumors in the 100- to 400-mm^3 size range (Table 2). At 2 h postinjection, however, there was less [^{18}F]FMISO in tumors (tumor-to-blood ratio = 1.40 ± 0.25) than was observed for [^{18}F]FETA (tumor-to-blood ratio = 2.20 ± 0.77, P = 0.001). Because [^{18}F]FETA blood levels peaked earlier than those for [^{18}F]FMISO (Fig. 3), [^{18}F]FETA may have distributed more quickly to the tumors that have low blood flow. The kidney-to-blood, intestine-to-blood and heart-to-blood ratios at both 2 and 4 h postinjection (Table 2) agree closely for the two tracers.

Differences in biodistribution of the two tracers suggest advantages for [^{18}F]FETA over [^{18}F]FMISO. Nitroimidazoles are metabolized principally in the liver and excreted mostly through the kidneys, with relatively less being cleared through the gastrointestinal tract (*19*). The retention of [^{18}F]FETA in the liver of mice is about half as high as that for [^{18}F]FMISO at 2 or 4 h after the intraperitoneal injection of tracer, whereas the levels of the two tracers are similar in the kidney (Table 2). However, the proportion of [^{18}F]FETA-derived radioactivity excreted in the urine as the parent drug, as opposed to metabolites, is greater at 2 or 4 h postinjection than is true for [^{18}F]FMISO (Table 3, Fig. 4). At 2 h after injection of [^{18}F]FETA, 32% of the radioactivity in the plasma is metabolites compared with 50% for [^{18}F]FMISO (Table 3). Fewer circulating metabolites for [^{18}F]FETA suggest that there may be less nonspecific backgrounds in well-oxygenated tissues.

We reanalyzed a large number of animal biodistribution studies performed with [^{18}F] or [^{3}H]FMISO, initially reported in Koh et al. (*6*). This reanalysis confirmed the results shown in Table 2 for uptake of [^{18}F]FMISO into various tissues. Although the lung is obviously well-oxygenated, we have consistently observed lung-to-blood ratios greater than 1.0 in mice but not other species (Fig. 5). Increased retention of [^{3}H]misonidazole in lung has been reported by Cobb et al. (*20*), who suggested that oxygen-independent nitroreductases may account for this increased uptake in lung and other presumably well-oxygenated tissues. Another possibility that deserves consideration is that metabolites of [^{18}F]FMISO also may be retained in the lung. The fact that [^{18}F]FMISO is metabolized more readily than [^{18}F]FETA (Table 3) is consistent with observations reported for these tracers in the lung. The lung-to-blood ratios for [^{18}F]FETA are close to 1.0 and are significantly lower than the uptake ratios for [^{18}F]FMISO at both 2 h (P = 0.001) and 4 h postinjection (P = 0.00003) (Table 2). These data, combined with those for liver uptake of the two tracers, support the conclusion that [^{18}F]FETA is metabolized less in vivo than is [^{18}F]FMISO.

Interestingly, the brain-to-blood ratio of [^{18}F]FETA is 1.07 ± 0.31 at 4 h postinjection, indicating that this radiopharmaceutical readily crosses the blood-brain barrier. The parent drug, etanidazole, has an octanol-water partition coefficient of 0.046, about 1/10th the partition coefficient of [^{18}F]FMISO (0.41) and largely is excluded from the brain, as determined by HPLC analysis after injection of an unlabeled drug (*17*). Replacement of the hydroxyl group in etanidazole with a fluorine atom is expected to increase the partition coefficient, and our observations of the uptake of [^{18}F]FETA into the brain are consistent with this expected change. These results demonstrating that [^{18}F]FETA readily enters the brain indicate that it can be used to image hypoxia in primary brain tumors or metastases to the brain from other sites, even if the blood-to-brain barrier remains intact at the edge of the tumor.

If [^{18}F]FETA proves to have advantages over [^{18}F]FMISO for in vivo imaging of tumors and consistently detects hypoxia across different tumor types, it still will be most useful for detecting extreme hypoxia, below 0.1% O_2 (1000 ppm) associated with resistance to radiation. Moderate hypoxia also may be important in tumors. For example, the vascular cytokine VEGF has been implicated in more aggressive tumor growth. Leith and Michelson (*21*) reported substantial secretion of VEGF at O_2 levels above anoxia in two colon carcinoma cell lines. One half of the maximum (log) increase in VEGF, obtainable with anoxic incubation,

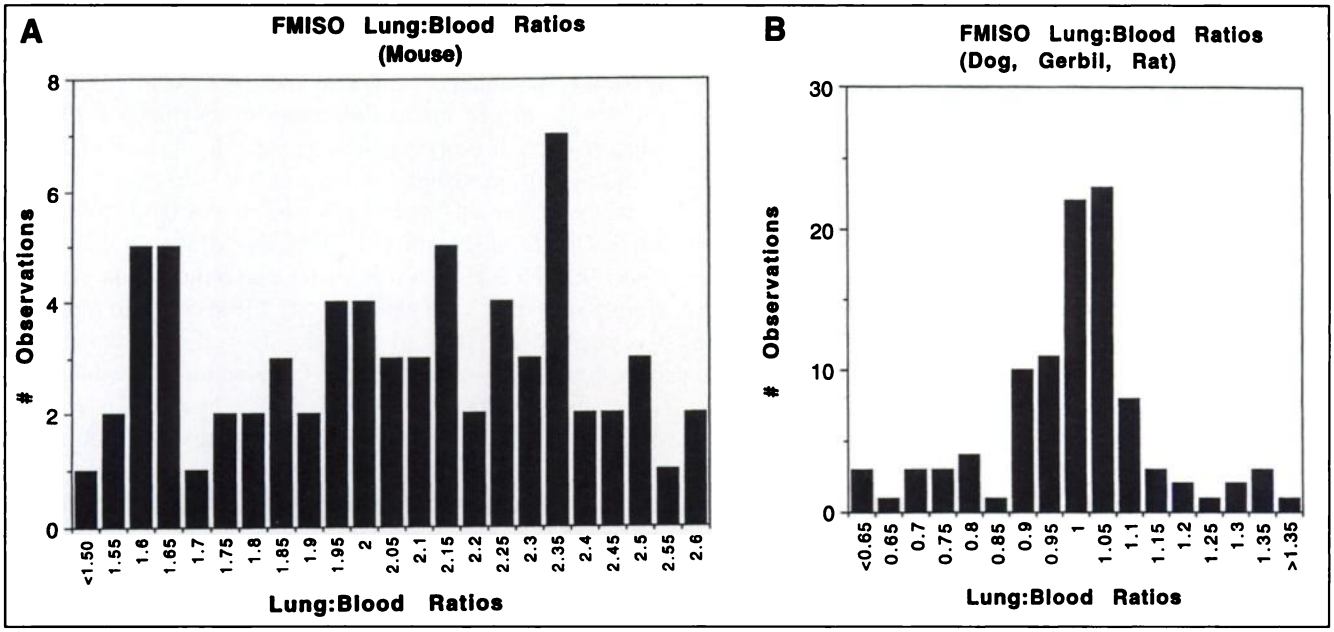

FIGURE 5. Lung-to-blood ratios for FMISO in four species, obtained from biodistribution studies with [^{18}F] or [^{3}H]FMISO. This figure represents reanalysis of data originally reported in Koh et al. (*6*). (A) Lung-to-blood ratios from mice at 2–4 h postinjection. (B) Lung-to-blood ratios from rats, dogs and gerbils 2–4 h after tracer injection.

occurred at 0.8% O_2 (8000 ppm) for clone A cells and 2.2% (2200 ppm) for HCT-8 cells. We also have observed that VEGF is induced in HT29 colon carcinoma and A549 lung carcinoma cells as effectively by exposure to 1000- or 5000-ppm O_2 as by contact with extreme hypoxia (*22*). At O_2 levels above 5000 ppm, [^{18}F]FMISO and [^{18}F]FETA binding is greatly reduced in most cell lines. Thus, it would be advantageous to synthesize additional radiolabeled probes for hypoxia that remain bound at O_2 levels slightly higher than those required for retention of [^{18}F]FETA or [^{18}F]FMISO. This may be achieved by placing substituents with different electron withdrawing or donating properties on the nitroaromatic ring, which in turn change the electron properties of the NO_2 group. This approach is based on the principle that nitroimidazoles are bioreductive alkylating agents, retained by a reaction more likely to occur in hypoxic cells because they reside at a lower redox potential than do oxic cells (*23*). The oxidized member of a redox pair, the unaltered parent drug, can be reduced by the reduced half of any redox pair at a more negative redox potential. This suggests that modifying the reduction potential of a drug will alter its ability to bind in cells with a particular redox environment. Specifically, we predict that withdrawing electrons from the NO_2 group will increase the O_2 level at which binding is inhibited by 50% (the K value). We plan to prepare etanidazole derivatives rather than derivatives of the more familiar FMISO because imidazoles substituted with electronegative groups have been shown to result in difficulties when reacted with epoxides. The reaction with epifluorohydrin and 2-nitroimidazole is the method by which the radioactive fluorine is introduced in the synthesis of FMISO (*7*). With FETA derivatives, the side chain will be put in place, which is likely to be the difficult step, before the radiofluorinated group is added (*11*); therefore, the difficult reaction will not have to be performed with the radioactive substrate. Such radiopharmaceuticals, if successfully synthesized, will be valuable for detecting and imaging more modest levels of hypoxia that have little effect on the response of tumors to radiation but may induce expression of genes associated with tumor aggressiveness.

CONCLUSION

[^{18}F]FETA demonstrates oxygen-dependent binding very similar to that for [^{18}F]FMISO when the two tracers are compared in vitro in the same cell lines. [^{18}F]FETA and [^{18}F]FMISO have similar retention in tumors 4 h after injection, but less [^{18}F]FETA is retained in liver and lung at both 2 and 4 h postinjection, and fewer [^{18}F]FETA metabolites are found in plasma and urine. The biodistribution pattern suggests [^{18}F]FETA may offer advantages for imaging of tumors in vivo because it is metabolized less readily.

ACKNOWLEDGMENT

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