Synthesis and Characterization of Lipophilic 1-[^{18}F]Fluoroalkyl-2-nitroimidazoles for Imaging Hypoxia

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In order to develop new imaging markers for brain hypoxia, two lipophilic nitroimidazoles, 1-(3-fluoropropyl)-2-nitroimidazole (FPN) and 1-(8-fluorooctyl)-2-nitroimidazole (FON) were synthesized and labeled with fluorine-18. The octanol/water partition coefficients were measured as an indication of lipophilicity, giving values of log P=0.28 for FPN and log P=2.72 for FON, respectively, which are in the range thought to be optimal for the diffusion of molecules across the blood-brain barrier. It was suggested from a comparative study of *in vitro* radiosensitization in V79 cells that these lipophilic analogs may have reduction potentials close to those of fluoromisonidazole (FMISO) and misonidazole (MISO), known hypoxic cell radiosensitizers. The preparation of ^{18}F-labeled FON (^{18}FON) and FPN (^{18}FPN) was achieved *via* two-steps through [^{18}F]fluoride ion displacement of tosylate precursors, in reasonable radiochemical yields. Tissue distribution of ^{18}FPN and ^{18}FON in normal rats and tumor-bearing mice after intravenous injection was investigated and compared to the behavior of ^{18}F-labeled FMISO (^{18}FMISO), a proven hypoxic imaging agent. The high lipophilicity of ^{18}FON and ^{18}FPN resulted in increased initial uptake into normal rat brain, relative to ^{18}FMISO, followed by a rapid washout from brain. Both of these lipophilic analogs had significantly lower tumor uptake and lower tumor-to-blood ratios than ^{18}FMISO, suggestive of a poor trapping mechanism within the tumor tissue. Neither ^{18}FON or ^{18}FPN offers improved biological properties over ^{18}FMISO as a potential agent for use in brain hypoxic imaging.

Key words nitroimidazole; fluorine-18; tissue biodistribution; tumor; brain

2-Nitroimidazole derivatives typified by misonidazole (MISO), originally developed as hypoxic radiosensitizers for radiation therapy, have been shown to be susceptible to reduction trapping in regions of low oxygen tension. The nitro group is believed to undergo a one-electron reduction in viable cells to produce a radical anion, while in hypoxic cells this intermediate is further reduced to species which react with cellular components and is hereby trapped within the cell. In normoxic conditions reoxidation rapidly takes place and the compound eventually diffuses out of the cell.[1—3]

There has been considerable interest in developing ^{99m}Tc, ^{123}I or ^{18}F-labeled compounds incorporating a 2-nitroimidazole moiety that may allow the visualization of hypoxic tissue *in vivo*.[4] ^{18}F-Labeled fluoromisonidazole (^{18}FMISO) has shown potential as an agent to map hypoxic tissue using positron emission tomography (PET).[5—8] However, this agent has drawbacks due to its relatively low concentration within hypoxic tissue and the necessity to wait a long time (2—4 h) to achieve acceptable target-background ratios for imaging. The target tissues have mostly been the myocardium or tumors, with less attention centered on the brain. The utility of ^{18}FMISO as a hypoxic imaging agent for brain studies appears to be limited because of its low blood-brain barrier (BBB) permeability.[9,10]

We have been interested in the development of new hypoxia PET markers exhibiting more rapid localization in hypoxic tissue with a greater BBB permeability than ^{18}FMISO, while avoiding excess nonspecific biodistribution. More lipophilic analogs would be expected to cross cellular membranes including the BBB more readily and result in a greater initial distribution to the target tissue than ^{18}FMISO, although with the risk of higher nonspecific distribution. Recently ^{99m}Tc-labeled nitroimidazole complexes with increased lipophilicity compared to ^{18}FMISO have been reported.[4,11] This article describes the synthesis of the two lipophilic 2-nitroimidazoles, 1-(3-fluoropropyl)-2-nitroimidazole (FPN) and 1-(8-fluorooctyl)-2-nitroimidazole (FON) and radiolabeling as ^{18}F-labeled analogs. Their *in vivo* biodistributions are also characterized in normal rats and in tumor-bearing mice to evaluate their potential use as markers of hypoxic tissue in comparison with ^{18}FMISO.

RESULTS AND DISCUSSION

Synthesis and Lipophilicity Numerous studies have demonstrated that lipophilicity is one of the highly significant contributors to brain penetration. The optimal octanol/water partition coefficient (log P) for brain penetration has been reported to be around 2 ± 0.5.[12,13] FPN and FON were the chosen target compounds, and were designed with a simple fluoroalkyl group based on log P calculations using the Pekker or Leo Hansch fragmental approach[14]: the calculated log P values lie in the $0.8<\log P<1.2$ range for FPN and in the $2.5<\log P<3.8$ range for FON.

FPN (**3**) and FON (**6**) were synthesized in two steps as shown in Chart 1. The starting compounds, di-tosyl octanate and di-tosyl propylate, were prepared by treatment of 1,8-octanediol and trimethylene glycol with *p*-toluenesulfonyl chloride in pyridine, respectively. The tosylate precursors (**2**) and (**5**) were obtained by coupling di-tosyl octanate and di-tosyl propylate with 2-nitroimidazole in the presence of triethylamine in *N*,*N*-dimethylformamide (DMF) under mild conditions, respectively. The preparations of FPN (**3**) and FON (**6**) were accomplished by fluoride substitution on the tosylate precursor (**2**) and (**5**) using tetrabutylammonium fluoride at room temperature in 84% and 89% yield respectively. For comparison purposes, FMISO was also prepared according to a literature procedure for radiosynthesis of ^{18}FMISO.[15]

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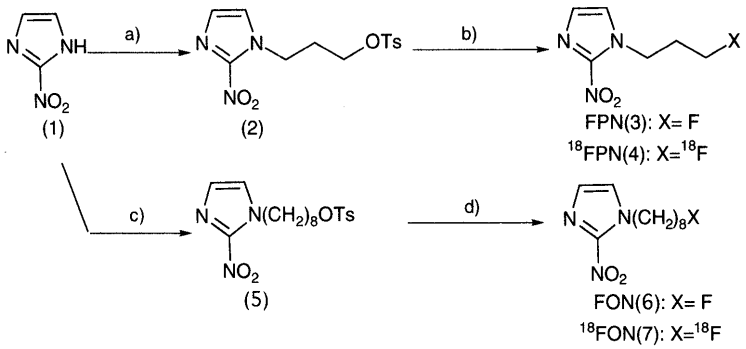
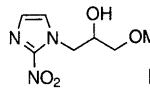
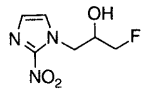
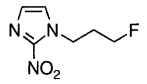
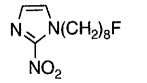


a) $TsO(CH_2)_3OTs$, Et_3N, DMF, r.t., 48 h; b) *n*-Bu_4NF, THF, r.t., 8 h or $K^{18}F$/Kryptofix 2.2.2. CH_3CN, 80℃, 10 min; c) $TsO(CH_2)_8OTs$, Et_3N, DMF, r.t., 48 h; d)*n*-Bu_4NF, THF, r.t., 6 d or $K^{18}F$/Kryptofix 2.2.2. CH_3CN, 80℃, 10 min

Chart 1

Table 1. Partition Coefficients of 2-Nitroimidazole Derivatives

Compound	log *P* (octanol/water)[a]
 MISO	−0.39[b]
 FMISO	−0.40 (−0.40[b])
 FPN	0.28
 FON	2.72

a) Octanol/water partition coefficient was determined by shaking solutions of compounds in 2.5 ml 1-octanol with 2.5 ml potassium phosphate buffer (pH 7.4) for 20 min and the data are the average of three separate experiments. *b*) Calculated from reported *P* values, ref. 25.

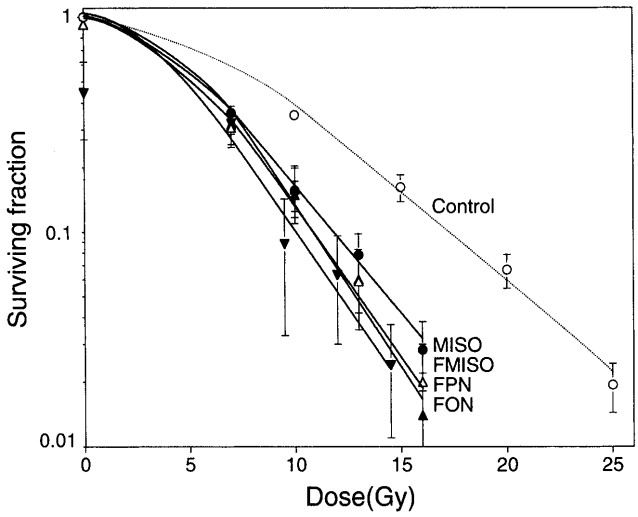


Fig. 1. Radiation Survival Curves of Hypoxic V79 Cells

Cells were incubated with 1 mM concentration of MISO (●), FMISO (△), FPN (▲) or FON (▼) immediately before X-ray irradiation under hypoxic conditions at room temperature, respectively. Each point represents the mean±S.E. for 3—6 experiments. Hypoxic control (○).

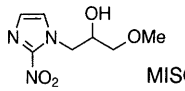
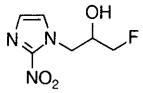
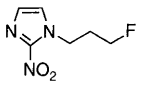
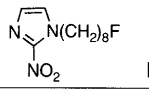
The structures of all compounds were confirmed by spectral methods.

The log *P* values of FPN and FON were thus measured as an index of lipophilicity by octanol/water extraction at pH 7.4, and are given in Table 1, in which for comparison the log *P* values for MISO and FMISO are also included. Greatly increased log *P* values for these two analogs, relative to MISO and FMISO, were observed and these analogs seemed to be lipophilic enough to freely enter the brain as supported by their high log *P* values.

Radiosensitization The reduction potential of the nitro group of a nitroimidazole is an important determinant of a compound's ability to progress along the pathway by which nitroimidazoles are selectively retained in hypoxic tissues. Therefore, reduction potential, in addition to lipophilic character, could be used as an additional indication of potential for imaging of hypoxic tissue. Hypoxic cell radiosensitizers are generally compounds with high electron affinity, and a correlation between electron affinities (one-electron reduction potentials) and radiosensitizing effectiveness with hypoxic cells has been reported to exist among nitroimidazoles, including MISO.[16—19] The radiosensitizing effect of FPN and FON on hypoxic V79 cells was thus investigated, comparing with those of MISO and FMISO as well-documented radiosensitizers. Since FPN and FON have low solubility in H_2O, all *in vitro* radiosensitization experiments were carried out in minimal essential medium (MEM) containing 1% dimethylsulfoxide (DMSO). The profiles of the dose–survival curves of Chinese hamster V-79 cells observed after X-ray irradiation with FPN and FON are shown in Fig. 1. An increase in the dose of the nitroimidazole agent caused a progressive increase in sensitization. It was found that both FPN and FON showed almost the same or only slightly greater sensitizing activity as MISO and FMISO at an equimolar concentration. The SER (sensitizer enhancement ratio) for FPN and FON were 1.81 and 1.75 respectively at a 1 mM concentration, in comparison to 1.44 for MISO and 1.60 for FMISO (Table 2). It was thus considered that there may be no significant differences in the reduction potentials of the four nitroimidazoles studied here.[20]

Radiosynthesis Radiochemical synthesis was achieved by nucleophilic displacement of a tosylate precursor with [^{18}F]fluoride ion. The no-carrier-added [^{18}F]fluoride activity obtained from the $^{18}O(p,n)^{18}F$ reaction was converted to $K^{18}F$/Kryptofix 2.2.2. as described earlier.[21] We found that optimal reaction conditions were a 10 min reaction time at

Table 2. Radiosensitizing Activity for Four 2-Nitroimidazoles

Compound	SER (1 mM)[a]
 MISO	1.44
 FMISO	1.60
 FPN	1.81
 FON	1.75

a) SER was calculated from the ratio of radiation doses for 10% survival with and without compound, by reading the curve in Fig. 1 for each sensitizer.

Table 3. Biodistribution of Radioactivity after ^{18}FMISO Injection in Normal Wistar Rats

Tissue	Uptake (% dose/g)[a]			
	5 min (n=5)	15 min (n=3)	30 min (n=4)	60 min (n=3)
Blood	0.373±0.121	0.403±0.052	0.450±0.040	0.379±0.005
Liver	0.475±0.292	0.664±0.100	0.939±0.220	0.727±0.040
Adrenals	0.402±0.242	0.449±0.056	0.800±0.129	0.395±0.051
Kidneys	0.433±0.235	0.523±0.114	0.743±0.133	0.645±0.082
Heart	0.411±0.151	0.429±0.053	0.503±0.057	0.411±0.018
Bone	0.076±0.061	0.112±0.051	0.171±0.059	0.109±0.033
Brain	0.127±0.080	0.235±0.060	0.390±0.076	0.370±0.025

a) Mean±S.D.

80 °C in acetonitrile, using the tosylates and Kryptofix 2.2.2.-potassium [^{18}F]fluoride complex. Total synthetic time of ^{18}FPN (**4**) and ^{18}FON (**7**) including HPLC purification were 50 and 60 min, respectively. The isolated radiochemical yields at the end of synthesis were 43% for ^{18}FPN (**4**) and 26% for ^{18}FON (**7**), respectively, with more than 96% radiochemical purity. ^{18}FMISO was also prepared in an 8% isolated radiochemical yield according to a modified literature method.[22] The specific activity was not determined but may be around 3700 GBq/mmol at the end of synthesis, as judged from the recent synthesis of other ^{18}F-labeled compounds in our laboratory.

***In Vivo* Biodistribution** Tissue distribution of the two ^{18}F-labeled fluoroalkyl-2-nitroimidazoles ^{18}FPN and ^{18}FON was determined in normal Wistar rats at 5, 15, 30 and 60 min following intravenous injection, including that of ^{18}FMISO for comparative purposes. At 5 min after injection, the blood concentration for each of these radiotracers was already at low levels, showing fast transport of the tracers to tissue. ^{18}FPN and ^{18}FON were cleared rather faster from blood relative to ^{18}FMISO.

^{18}FMISO showed the highest uptake by the liver followed by the kidney as shown in Table 3. The time of maximum uptake in most organs was 30 min postinjection and the radioactivity, thereafter, cleared slowly or remained constant between 30 and 60 min after injection. ^{18}FMISO displayed an early peak in brain uptake (30 min) followed by relatively slow washout, with brain-to-blood ratios being less than unity during the first 60 min. These results are qualitatively similar to those previously reported for ^{18}FMISO using Mature Sprague-Dawley rats.[6]

Table 4. Biodistribution of Radioactivity after ^{18}FPN Injection in Normal Wistar Rats

Tissue	Uptake (% dose/g)[a]			
	5 min	15 min	30 min	60 min
Blood	0.463±0.049	0.361±0.044	0.306±0.032	0.152±0.004
Liver	1.083±0.216	1.231±0.393	1.277±0.374	1.250±0.090
Adrenals	0.562±0.036	0.454±0.076	0.391±0.033	0.215±0.043
Kidneys	0.787±0.136	0.866±0.165	0.835±0.096	0.622±0.039
Heart	0.506±0.041	0.386±0.068	0.634±0.063	0.146±0.011
Bone	0.093±0.044	0.117±0.029	0.106±0.004	0.178±0.036
Brain	0.480±0.043	0.343±0.093	0.264±0.053	0.087±0.007

a) Mean±S.D. of three rats.

Table 5. Biodistribution of Radioactivity after ^{18}FON Injection in Normal Wistar Rats

Tissue	Uptake (% dose/g)[a]			
	5 min	15 min	30 min	60 min
Blood	0.346±0.083	0.351±0.049	0.267±0.042	0.191±0.026
Liver	1.338±0.894	1.192±0.275	0.990±0.085	0.570±0.057
Adrenals	0.997±0.526	0.748±0.061	0.507±0.070	0.251±0.042
Kidneys	1.209±0.712	1.390±0.243	1.061±0.197	0.561±0.129
Heart	0.484±0.176	0.398±0.057	0.262±0.035	0.169±0.028
Bone	0.136±0.101	0.375±0.055	1.063±0.276	1.340±0.263
Brain	0.433±0.206	0.307±0.031	0.190±0.027	0.128±0.026

a) Mean±S.D. of three rats.

The extent of defluorination for ^{18}FPN, indicated by bone radioactivity, was low over time as shown in Table 4 and is comparable to that for ^{18}FMISO, suggesting that *in vivo* metabolites of ^{18}FPN does not result in the liberation of the fluoride ion. In contrast, unfavorable high bone radioactivity was observed with ^{18}FON which reached about 24% of the injected dose at 60 min postinjection, indicating that ^{18}FON is susceptible to metabolic defluorination.

A biological characteristic of ^{18}FPN and ^{18}FON is that both liver and kidneys showed higher uptake than most other normal tissues (Tables 4, 5) as observed with ^{18}FMISO, and the uptake levels were much higher than those observed with ^{18}FMISO. It has been reported that metabolism of nitroimidazoles occurs in the liver and excretion occurs principally through the kidney.[23,24] The liver was the organ with greatest accumulation of ^{18}FPN and radioactivity remained fairly constant from 5 to 60 min postinjection, suggesting an accumulation of labeled metabolites. On the other hand, the initial high liver uptake of radioactivity for ^{18}FON decreased rapidly with time. This may be in part the result of metabolic defluorination of ^{18}FON as evident from progressive accumulation of radioactivity in the bone.

Brain uptake as a function of time after injection is depicted graphically in Fig. 2. It is seen that ^{18}FON and ^{18}FPN exhibited a similar time course of uptake and elimination from the brain. The initial brain radioactivity was significantly higher for ^{18}FPN and ^{18}FON than for ^{18}FMISO, indicating higher brain penetration than ^{18}FMISO, as expected. It is also suggested that the maximal brain uptake of these two ^{18}F-analogs may have occurred at less than 5 min after injec-

tion. It should be noted that rapid washout of radioactivity from the brain was observed, in parallel to the radioactivity in the blood, with no indication of accumulation. Such an uptake and washout pattern of radioactivity observed in the normal brain thus appeared to be a desirable characteristic as a potential agent for imaging the hypoxic tissue of brain. The regional distributions of radioactivity in brain were uniform for all three ^{18}F-labeled nitroimidazoles at all time points examined (data not shown).

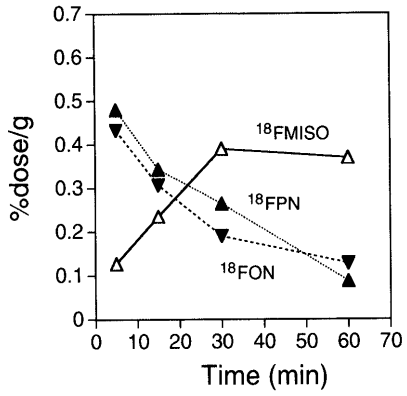


Fig. 2. Brain Uptake of Three ^{18}F-Fluorinated Nitroimidazoles at Different Times after i.v. Injection

Data are the average from three animals.

It is generally known that many solid tumors contain areas of diminished oxygen supply.[1,25] Biodistribution of ^{18}FON and ^{18}FPN at 30 and 60 min postinjection in C3H mice bearing two different transplanted tumors (methylcholanthrene induced fibrosarcoma and squamous carcinoma) as rodent tumor models, was further investigated to assess the accumulation of the tracers in tumors. The binding of ^{18}FMISO to hypoxic cells has been demonstrated mainly in *in vitro*-studies[7,8] and PET studies of patients with cancer have shown positive imaging of various tumors.[4] Therefore, for comparison, a biodistribution study of ^{18}FMISO was also carried out in the same tumor-bearing mice. Both tumor cells were implanted in the right or left leg muscle of C3H mouse and allowed to grow for 9—10 d.

Tumor load did not significant influence the uptake of these three ^{18}F-labeled analogs in normal organs. The more

Table 6. Biodistribution of ^{18}FMISO in Tumor Bearing C3H Mice

Tissue	Uptake (% dose/g)[a]			
	Fibrosarcoma		Squamous Carcinoma	
	30 min (n=4)	60 min (n=4)	30 min (n=4)	60 min (n=4)
Blood	3.742±0.331	3.038±0.171	4.102±0.570	3.559±0.608
Lung	4.311±0.276	3.868±0.301	4.488±1.043	4.495±0.592
Liver	7.374±0.766	6.575±0.518	6.766±1.083	7.157±0.823
Small intestine	4.985±0.397	4.157±0.906	5.225±0.375	5.062±1.104
Adrenals	2.465±0.238	2.357±0.373	2.729±0.954	1.928±0.522
Kidneys	6.170±0.468	4.710±0.545	5.642±0.712	5.278±0.998
Heart	3.906±0.443	3.304±0.151	4.410±0.645	3.643±0.341
Tumor	3.819±0.282*	4.760±0.925**	4.093±0.643	4.888±0.746*
Muscle	3.334±0.195	2.749±0.221	3.703±0.456	3.246±0.980
Bone	0.820±0.222	1.360±0.209	1.453±0.558	1.454±0.143
Brain	3.320±0.226	3.034±0.148	3.799±0.548	3.288±0.451
Tumor/blood	1.022±0.036	1.582±0.390	1.004±0.148	1.409±0.370
Tumor/muscle	1.145±0.033	1.721±0.203	1.112±0.175	1.608±0.500

a) Mean±S.D. t-test: $*p<0.05$, $**p<0.01$.

Table 7. Biodistribution of ^{18}FPN in Tumor Bearing C3H Mice

Tissue	Uptake (% dose/g)[a]			
	Fibrosarcoma		Squamous Carcinoma	
	30 min (n=4)	60 min (n=3)	30 min (n=4)	60 min (n=4)
Blood	2.580±0.552	2.026±0.110	2.673±0.375	1.838±0.345
Lung	2.925±0.939	2.806±0.524	4.979±0.727	3.514±1.083
Liver	18.148±2.872	13.148±1.671	18.792±2.368	17.322±5.221
Small intestine	14.062±5.922	3.232±1.390	11.346±4.028	3.897±0.622
Adrenals	1.907±0.678	1.826±0.528	2.073±0.284	1.313±0.343
Kidneys	7.240±0.792	4.034±0.342	9.456±0.984	5.103±0.975
Heart	1.762±0.499	1.400±0.127	1.685±0.296	1.357±0.410
Tumor	1.504±0.346	1.593±0.084*	1.565±0.283	1.683±0.703
Muscle	1.131±0.140	1.166±0.123	1.211±0.225	1.070±0.252
Bone	2.797±0.789	2.804±0.828	1.982±0.169	5.551±1.385
Brain	0.611±0.088	0.586±0.091	0.725±0.198	0.523±0.125
Tumor/blood	0.583±0.053	0.788±0.062	0.583±0.029	0.893±0.219
Tumor/muscle	1.318±0.167	1.375±0.130	1.294±0.025	1.532±0.279

a) Mean±S.D. t-test: $*p<0.01$.

Table 8. Biodistribution of ^{18}FON in Tumor Bearing C3H Mice

Tissue	Uptake (% dose/g)[a]			
	Fibrosarcoma		Squamous Carcinoma	
	30 min (n=4)	60 min (n=4)	30 min (n=3)	60 min (n=4)
Blood	3.453±1.745	1.517±0.158	2.812±0.749	1.495±0.119
Lung	14.649±2.843	4.571±0.654	16.621±5.677	6.262±0.873
Liver	6.453±0.387	3.426±0.342	5.821±1.833	4.268±1.487
Small intestine	4.724±2.194	2.528±0.251	5.713±1.973	2.450±0.488
Adrenals	1.545±0.233	1.065±0.347	1.681±0.392	0.934±0.145
Kidneys	5.519±0.479	2.833±0.268	6.609±1.822	2.990±0.101
Heart	2.396±0.091	1.531±0.183	2.480±0.583	1.440±0.087
Tumor	2.136±0.124***	1.542±0.213**	2.798±0.559*	1.794±0.158***
Muscle	1.478±0.102	0.933±0.216	1.737±0.349	0.904±0.090
Bone	12.509±2.529	11.920±2.067	9.926±1.529	16.461±1.419
Brain	1.218±0.048	1.082±0.080	1.173±0.197	1.083±0.054
Tumor/blood	0.704±0.232	1.014±0.045	1.035±0.289	1.210±0.185
Tumor/muscle	1.446±0.033	1.708±0.395	1.635±0.311	2.002±0.292

a) Mean±S.D. t-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

lipophilic nature of ^{18}FON is represented by higher accumulation in the lung. As seen in Tables 6—8 both ^{18}FON and ^{18}FPN showed lower uptake of radioactivity in fibrosarcoma as compared to ^{18}FMISO, although the tumor radioactivity remained essentially constant between two time points.

The tumor-to-blood ratios for ^{18}FPN and ^{18}FON, shown in Fig. 3, increased with time but lower than those observed with ^{18}FMISO, although these derivatives did show somewhat higher tumor-to-muscle ratios. Squamous carcinoma tumors showed absolute uptake in tumor and tumor-to-blood ratios and tumor-to-muscle ratios not significantly different from those observed with fibrosarcoma tumors. It is reasonable to assume that the uptake in tumors of ^{18}FON and ^{18}FPN is related to hypoxia, in analogy with other 2-nitroimidazoles. This study thus indicated poor localization of ^{18}FON and ^{18}FPN in hypoxic tumors, suggestive of as the consequence of a poor trapping mechanism in tumor hypoxia. However, the high lipophilic nature of ^{18}FPN and ^{18}FON may also be considered as a reason, causing reduced delivery of the tracers to the tumor tissue due to increased non-specific trapping of these agents within the reticuloendothelial system, as characterized by the high levels of hepatic accumulation.

An optimal hypoxic imaging agent should localize in hypoxic tissue quickly with a good target/nontarget ratio in a short time while minimizing nonspecific distribution. Comparison with the *in vivo* behavior of ^{18}FMISO in both the mouse-tumor system and normal rats indicates the less favorable properties of the lipophilic ^{18}FON and ^{18}FPN as candidates for imaging the states of hypoxia in brain. While the entry of a compound into tissue is a function of its partition coefficient, reduction potential is responsible for the ability to undergo bioreduction resulting in trapping within hypoxic tissue. Neither one of these lipophilic analogs appears to be far from the ideal reduction potential which enables efficient hypoxia-mediated binding.

The basic structure of nitroimidazoles is considered to be two functional parts: the nitroimidazole ring is very important for the electron affinity and reductive properties, and the N-1 side chain determines the pharmacokinetics. The N-1 side chain of MISO is known to be retained during covalent

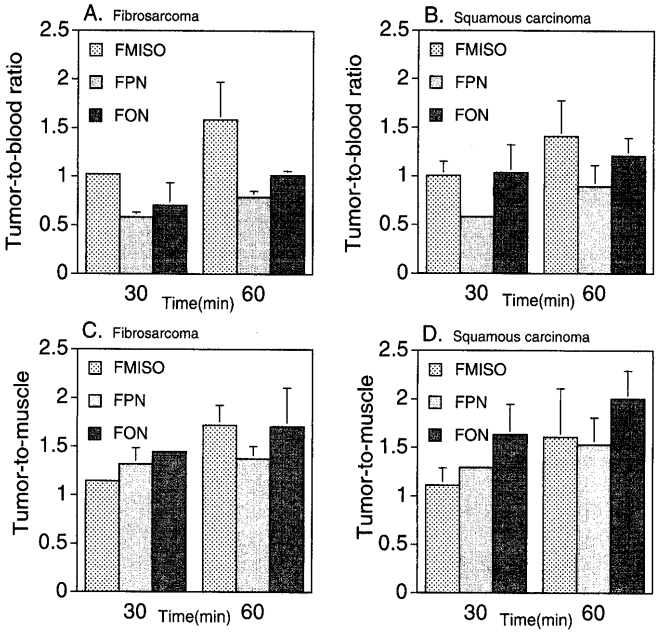


Fig. 3. Tumor-to-Blood and Tumor-to-Muscle Ratios of Three ^{18}F-Fluoronitroimidazole in Fibrosarcoma (Panel A, C) and Squamous Carcinoma (Panel B, D)-Bearing Mice

Data are extracted from Tables 6—8.

binding to macromolecules.[26,27] It has also been reported that ^{18}F-fluoronorhydroxy metronidazole showed no selective uptake in rat brain hypoxic tissue. A hydroxy group β to the imidazole may be required for the preferential uptake and binding of 2-nitroimidazoles in hypoxic tissue, as has been pointed out by Welch and co-workers.[9]

It is likely that derivatives based on 2-nitroimidazole, both with optimal lipophilicity for passing the BBB and more rapid oxygen-sensitive bioreduction, merit further investigation for use in brain imaging. Based on the findings of this study, we are continuing to refine our design of this class of compounds for development of hypoxia-targeting radiopharmaceuticals.

EXPERIMENTAL

Unless otherwise stated, chemical reagents were obtained from commercial sources and were used directly. All melting points are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL GX-270 spectrometer (270 MHz) and the chemical shifts are reported in parts per million downfield from tetramethylsilane. Infrared (IR) spectra were recorded with a JASCO IR Report-100 spectrometer. Mass spectra were obtained with a JEOL JMS DX-610 or SX-102 mass spectrometer. Column chromatography was performed on Kieselgel 60 (70—230 mesh, Merck) and analytical TLC was carried out on Silica gel 60F 254 (Merck). In the synthetic procedures, organic extracts were routinely dried over anhydrous Na_2SO_4 and evaporated with a rotary evaporator under reduced pressure. Analysis of radioactivity on TLC plates was performed with a Aloka radiochromatogram scanner. HPLC was done using a Tosoh CCP & 8020 series HPLC system fitted with a Whatman Partisil 5PAC (10×100 mm) with monitoring of the radioactivity as well as UV absorption (at 254 nm). The radioactivity was also quantified with a Capintec radioisotope calibrator (CRC-30). The identity of radiolabeled compounds was supported by HPLC co-injection studies. MISO was supplied by Hoffman-La Roche Ltd.

Fluorine-18 was produced from 8 or 16% enriched [^{18}O]H_2O by the ^{18}O(p, n)^{18}F reaction as described previously.[28] Aminopolyether (Kryptofix 2.2.2.) supported potassium [^{18}F]fluoride (K^{18}F/Kryptofix) was prepared by the addition of $K_2CO_3 \cdot 1.5H_2O$ (1.0 mg) and Kryptofix 2.2.2. (3.0 mg) to irradiated water in a TPX (polymethylpentene) vessel and subsequent removal of the water under a stream of argon at 110 °C by azeotropic distillation with dry CH_3CN. Radiochemical yields were expressed at the end-of-synthesis (not corrected for decay) relative to the amount of the [^{18}F]fluorinating agent measured as total radioactivity present in the reaction vessel. All animal experiments were carried out in accordance with the regulations on animal experiments of the Faculty of Pharmaceutical Sciences, Kyushu University.

FMISO To a solution of 2-nitroimidazole (**1**) (75 mg, 0.66 mmol) in CH_3CN–H_2O (1 : 1, 4 ml) was added epifluorohydrin (151 mg, 1.99 mmol) and $NaHCO_3$ (206 mg, 2.45 mmol). The mixture was placed in a screw-capped vial and sealed. The mixture was stirred at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was extracted with CH_2Cl_2 (3 ml×3). The organic layer was filtered through Na_2SO_4 (30 mg) and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (EtOAc) to afford FMISO (98.3 mg, 78%) as a pale yellow powder, mp 148—152 °C (lit.[29] 139.5—140 °C). ^{1}H-NMR (methanol-d_4) δ: 7.45 (d, 1H, J=1.3 Hz, NC<u>H</u>=CHN), 7.14 (d, 1H, J=1.3 Hz, NC<u>H</u>=CHN), 4.74 (dd, 1H, J=3.6, 13.9 Hz, NC<u>H</u>$_2$CHOHCH$_2$F), 4.452 (dd, 1H, J=8.6, 13.9 Hz, NC<u>H</u>$_2$CHOHCH$_2$F), 4.447 (dd, 2H, J=4.3, 47.2 Hz, NCH$_2$CHOHC<u>H</u>$_2$F), 4.14 (dddt, 1H, J=3.6, 4.3, 8.6, 20.2 Hz, NCH$_2$C<u>H</u>OHCH$_2$F). IR (Nujol): 3300, 1540, 1280, 1160, 1120 cm^{-1}. FAB-MS *m/z*: 190 (MH$^+$). HR-MS (FAB-MS) Calcd for $C_6H_9FN_3O_3$: 190.0628. Found: 190.0632.

1,3-Bis(*p*-toluenesulfonyloxy)propane To an ice-cooled (0 °C) solution of *p*-toluenesulfonyl chloride (7.52 g, 39.4 mmol) in dry pyridine (5 ml) was added dropwise a solution of trimethylene glycol (1.00 g, 13.1 mmol) in pyridine (5 ml). The mixture was stirred at the same temperature for 3 h and poured into ice-cooled water (50 ml), and extracted with EtOAc (50 ml×3). The organic layer was dried and evaporated to dryness. The residue was chromatographed on silica gel (hexane : EtOAc=2 : 1 to 1 : 1) to afford the ditosylate (4.98 g, 99%) as a colorless crystalline solid, mp 88—90 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.76 (d, 4H, J=8.3 Hz, Ar-<u>H</u>), 7.33 (d, 4H, J=8.5 Hz, Ar-<u>H</u>), 4.07 (t, 4H, J=6.1 Hz, TsOC<u>H</u>$_2$), 2.46 (s, 6H, Ar-C<u>H</u>$_3$), 2.05 (dt, 2H, J=6.0, 6.1 Hz, TsOCH$_2$C<u>H</u>$_2$CH$_2$OTs). IR (Nujol): 2925, 1600, 1365 cm^{-1}.

1-(3-*p*-Toluenesulfonyloxypropyl)-2-nitroimidazole (2) Under argon, to a solution of 1,3-bis(*p*-toluenesulfonyloxy)-propane (1.36 g, 3.55 mmol) in dry DMF (2 ml) containing triethylamine (362 mg, 3.56 mmol) was added a solution of 2-nitroimidazole (100.4 mg, 0.89 mmol) in dry DMF (2 ml). The mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with EtOAc (60 ml) and washed with water (10 ml×2). The organic layer was dried and evaporated to dryness. The residue was chromatographed on silica gel (hexane : EtOAc=1 : 1) to afford 1-(3-*p*-toluenesulfonyloxypropyl)-2-nitroimidazole (**2**) (189.1 mg, 65%) as colorless needles, mp 107—109 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.79 (d, 2H, J=8.6 Hz, Ar-<u>H</u>), 7.38 (d, 2H, J=7.9 Hz, Ar-<u>H</u>), 7.12 (d, 1H, J=1.0 Hz, N–C<u>H</u>=CH–N), 7.09 (d, 1H, J=1.0 Hz, N–C<u>H</u>=CH–N), 4.52 (t, 2H, J=6.6 Hz, NC<u>H</u>$_2$CH$_2$-CH$_2$OTs), 4.04 (t, 2H, J=5.6 Hz, NCH$_2$CH$_2$C<u>H</u>$_2$OTs), 2.47 (s, 3H, Ar-C<u>H</u>$_3$), 2.24 (m, 2H, NCH$_2$C<u>H</u>$_2$CH$_2$OTs). IR (Nujol): 2950, 1595, 1535, 1485, 1360 cm^{-1}. Field Desorption (FD)-MS *m/z*: 325 (M$^+$).

FPN (3) Under argon, to a solution of 1-(3-*p*-toluenesulfonyloxypropyl)-2-nitroimidazole (**2**) (169 mg, 0.519 mmol) in dry tetrahydrofuran (THF, 4 ml) was added dropwise a 1 M-THF solution of *n*-Bu$_4$NF (2.08 ml, 2.08 mmol as F$^-$). The mixture was stirred at room temperature for 24 h and evaporated to dryness. The residue was chromatographed on silica gel (EtOAc : Hexane=1 : 1) to give FPN (**3**) (85.1 mg, 95%) as a pale yellow powder, mp 73—75 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.18 (d, 1H, J=1.0 Hz, NC<u>H</u>=CHN), 7.14 (d, 1H, J=1.0 Hz, NC<u>H</u>=CHN), 4.60 (t, 2H, J=6.6 Hz, NC<u>H</u>$_2$CH$_2$-CH$_2$F), 4.49 (dt, 2H, J=5.5, 45.9 Hz, NCH$_2$CH$_2$C<u>H</u>$_2$F), 2.27 (dtt, 2H, J=5.5, 6.6, 27.4 Hz, NCH$_2$C<u>H</u>$_2$CH$_2$F). IR (Nujol): 3400, 3160, 2970, 1875, 1700, 1530 cm^{-1}. EI-MS *m/z*: 173 (M$^+$). HR-MS (FAB-MS). Calcd for $C_6H_9FN_3O_2$: 174.0679 (MH$^+$). Found: 174.0636.

1,8-Bis(*p*-toluenesulfonyloxy)octane To an ice-cooled (0 °C) solution of 1,8-octanediol (2.02 g, 13.8 mmol) in dry pyridine (8 ml) was added dropwise an ice-cooled (0 °C) solution of *p*-toluenesulfonyl chloride (10.5 g, 55.1 mmol) in dry pyridine (12 ml). The reaction mixture was stirred at the same temperature for 4 h and poured into ice-cooled water (200 ml). The precipitate was then filtered and dried *in vacuo*. The crude solid was recrystallized from EtOAc to afford 1,8-bis(*p*-toluenesulfonyloxy)octane (5.89 g, 58%) as colorless needles, mp 71—73 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.87—7.27 (m, 8H, Ar-<u>H</u>), 4.02 (t, 4H, J=6.5 Hz, TsOC<u>H</u>$_2$), 2.46 (s, 6H, Ar-C<u>H</u>$_3$), 2.30—1.10 (m, 12H, TsOCH$_2$(C<u>H</u>$_2$)$_6$-CH$_2$OTs). IR (nujol): 2920, 2850, 1590 cm^{-1}. FD-MS *m/z*: 455 (MH$^+$).

1-(8-*p*-Toluenesulfonyloxyoctyl)-2-nitroimidazole (5) Under argon, to a solution of 1,8-bis(*p*-toluenesulfonyloxy)-octane (1.63 g, 3.59 mmol) in dry DMF (3 ml) containing tri-

ethylamine (557 mg, 5.47 mmol) was added a solution of 2-nitroimidazole (100.0 mg, 0.88 mmol) in dry DMF (2 ml). The mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with EtOAc (80 ml) and washed with water (10 ml×2). The organic layer was dried and evaporated to dryness. The residue was chromatographed on silica gel (hexane : EtOAc=1 : 1) to afford 1-(8-*p*-toluenesulfonyloxyoctyl)-2-nitroimidazole (**5**) (236 mg, 68%) as colorless needles, mp 48—50 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.81—7.76 (m, 2H, Ar-H), 7.37—7.33 (m, 2H, Ar-H), 7.09 (d, 2H, J=1.0 Hz, N-CH=CH-N), 4.39 (t, 2H, J=7.3 Hz, $NCH_2(CH_2)_6CH_2OTs$), 4.02 (t, 2H, J=6.6 Hz, $NCH_2(CH_2)_6CH_2OTs$), 2.45 (s, 3H, Ar-CH_3), 1.86—1.80 (m, 2H, $NCH_2(CH_2)_6CH_2OTs$), 1.64—1.57 (m, 4H, $NCH_2(CH_2)_6CH_2OTs$), 1.34—1.24 (m, 6H, $NCH_2(CH_2)_6CH_2OTs$). IR (Nujol): 3400, 2950, 2850, 1535, 1482, 1355 cm^{-1}. FAB-MS *m/z*: 396 (MH^+).

FON (6) Under argon, to a solution of 1-(8-*p*-toluenesulfonyloxyoctyl)-2-nitroimidazole (**5**) (85.8 mg, 0.217 mmol) in dry THF (2 ml) was added dropwise a 1 M-THF solution of *n*-Bu_4NF (0.87 ml, 0.87 mmol as F^-). The mixture was stirred at room temperature for 6 d. The reaction mixture was evaporated to dryness and chromatographed on silica gel (EtOAc : hexane=2 : 1 to EtOAc) to yield FON (**6**) (47.2 mg, 89%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.15 (d, 1H, J=1.0 Hz, NCH=CHN), 7.08 (d, 1H, J=1.0 Hz, NCH=CHN), 4.41 (t, 2H, J=7.3 Hz, $NCH_2(CH_2)_6CH_2F$), 4.44 (dt, 2H, J=6.1, 47.2 Hz, $NCH_2(CH_2)_6CH_2F$), 1.88—1.83 (m, 2H, $NCH_2(CH_2)_6CH_2F$), 1.76—1.57 (m, 4H, $NCH_2(CH_2)_6CH_2F$), 1.36 (m, 6H, $NCH_2(CH_2)_6CH_2F$). IR (Nujol): 2920, 2850, 1720, 1535, 1480 cm^{-1}. FAB-MS *m/z*: 244 (MH^+). HR-MS (FAB-MS). Calcd for $C_{11}H_{19}FN_3O_2$: 244.1461 (MH^+). Found: 244.1462.

Partition Coefficients The log *P* values were measured using a standard shake flask method. The samples were well shaken with a mixture of 1-octanol (2.5 ml) and 0.05 M phosphate buffer (2.5 ml, pH 7.4) for 20 min at 25 °C, after which aliquots of both phases were taken for analysis by HPLC quantitation. The reported log *P* values represent the mean of three experiments.

Radiosensitization Radiosensitization studies were carried out by employing asynchronous monolayer cultures of Chinese hamster V-79 cells. The V79 cells were cultured as monolayers in MEM supplemented with 10% fetal bovine serum. The nitroimidazoles were dissolved in MEM including 1% DMSO to a stock concentration of 2 mM and the appropriate volume of the nitroimidazole-containing solution was added to the cultures.

The radiation used was 200 kVp X-rays with 0.3 mm copper filtration at a dose rate of 1.49 Gy/min. All experiments were carried out in sealed glass test-tubes, gassed at room temperature with humidified nitrogen or air for oxic controls. The cells rounded up and became detached from the culture dish. To exclude the influence of morphological changes on cellular radiosensitivity, exponentially growing cells were irradiated at room temperature in suspension, which were trypsinized and suspended in fresh medium (1×10^5 cells/ml) just before X-irradiation, and 1 mM concentrations of MISO, FMISO, FPN or FON in the medium was added to the cells immediately before X-ray irradiation under hypoxic conditions at room temperature, respectively. Cell survival was determined using a conventional colony-forming assay. After postirradiation treatment, the test compound was removed by two washes with fresh medium. Subsequently, cells were counted, diluted, and plated on culture dishes. After incubation for 7 d, the colonies were then fixed with 10% formalin solution saturated with $MgCO_3$, stained with crystal violet, and then counted, and survival curves were then constructed. All experiments were performed at least in triplicate. Radiosensitization was measured as the mean of the SER calculated at 10% survival level.

^{18}FMISO (1-(3-[^{18}F]Fluoro-2-hydroxypropyl-2-nitroimidazole) (2*R*)-(−)-Glycidyl tosylate (4 mg, 17.5 μmol) dissolved in dry CH_3CN (300 μl) was added to a TPX vessel containing the $K^{18}F$/Kryptofix (40—70 MBq). The vessel was closed tightly and heated in an oil bath at 95 °C for 15 min, and then ice-cooled to 0 °C. The reaction mixture was filtered through silica gel (50 mg) with CH_3CN (2 ml). To the filtrate was added 2-nitroimidazole (2 mg, 17.7 μmol), $NaHCO_3$ (3.5 mg, 41.6 μmol) and water (1 ml). The vessel was sealed and heated at 110 °C for 50 min. After cooling to room temperature, the reaction mixture was extracted with CH_2Cl_2 (2 ml×3), dried and evaporated *in vacuo*. The residue was injected into a HPLC apparatus (Whatman Partisil 5PAC, 10×100 mm, $CHCl_3$: MeOH=59 : 1, 2 ml/min). The radioactive fraction eluting at t_R=7.0 min, corresponding to authentic FMISO, was collected. Total synthetic time was 110 min. The radiochemical yield (not corrected for decay) was 8.0%. No radiochemical impurities were detected by HPLC.

^{18}FPN (4) 1-(3-*p*-Toluenesulfonyloxypropyl)-2-nitroimidazole (**2**) (1 mg, 3.05 μmol) dissolved in dry CH_3CN (300 μl) was added to a TPX vessel containing the $K^{18}F$/Kryptofix 222 (40—70 MBq). The vessel was closed and heated in an oil bath at 80 °C for 10 min, and then cooled to room temperature. The reaction mixture was filtered through Sep-Pak-Silica with EtOAc (2 ml). The filtrate was evaporated *in vacuo*. The residue was dissolved in hexane : EtOAc=1 : 1 (1 ml) and injected into the HPLC (Whatman Partisil 5PAC, 10×100 mm, hexane : EtOAc=1 : 1, 2 ml/min). The radioactive fraction eluting at t_R=9.0 min, corresponding to authentic FPN was collected. Total synthetic time was 50 min. The radiochemical yield (not corrected for decay) was 43%. No radiochemical impurities were detected by HPLC.

^{18}FON (7) 1-(8-*p*-Toluenesulfonyloxyoctyl)-2-nitroimidazole (**5**) (1 mg, 2.52 mmol) dissolved in dry CH_3CN (300 μl) was added to a TPX vessel containing the $K^{18}F$/Kryptofix 222 (40—75 MBq). The vessel was closed and heated in an oil bath at 80 °C for 10 min, and then cooled to room temperature. The reaction mixture was filtered through Sep-Pak-Silica with EtOAc (2 ml). The filtrate was evaporated *in vacuo*. The residue was dissolved in hexane : EtOAc=2 : 1 (1 ml) and purified by HPLC (Whatman Partisil 5PAC, 10×100 mm, hexane : EtOAc=2 : 1, 2 ml/min), to give radiochemically pure ^{18}FON (**7**) (t_R=9.0 min) in 26% radiochemical yield (total time for synthesis, 60 min).

Biodistribution in Normal Rats Male Wistar rats (7 weeks old, 200—230 g) given standard laboratory food and water *ad libitum* were used in this investigation. Aliquots of ^{18}FMISO, ^{18}FPN or ^{18}FON in about 300 μl of saline solution,

with activities ranging from 0.4—1.6 MBq, were injected through the tail vein of unanesthetized rats. After given time intervals the animals were killed by cervical dislocation while under ether anesthesia. All samples of either the blood or the organ of interest that had been blotted free of blood, were taken and weighed. The brain was rapidly removed and dissected into regions of interest. The radioactivity of all samples was then measured with a Packard Auto-Gamma 500 scintillation counter (corrected for decay) and the percent injected dose per gram (% dose/g) of tissue weight was then calculated for each type of tissue.

Biodistribution in Tumor-Bearing Mice 3-Methylcholanthrene-induced fibrosarcoma (NFSa) or squamous carcinoma (SCCVII) was inoculated s.c. into the right or left hind leg muscle of female C3H/He mice (5 weeks old). The tumors which developed with a diameter of about 1 cm at 9—10 d after inoculation were used.[21] The animals were allowed free access to water and food at all times. Aliquots of ^{18}FMISO, ^{18}FPN or ^{18}FON in about 300 μl of saline solution, with activities ranging from 0.4—1.6 MBq, were injected through the tail vein of unanesthetized mice (18—20 g). At 30 and 60 min postinjection, the animals were killed by cervical dislocation while under ether anesthesia. Samples of blood and the tissues of interest, blotted free of blood, were taken, weighed, and assayed for radioactivity in a Packard Auto-Gamma 500 scintillation counter (corrected for decay). The results are expressed as percent injected dose per gram (% dose/g) of tissue weight, and tumor-to-blood and tumor-to-muscle concentration ratios were calculated from the % dose/g tissue data.

Statistical Analysis All values of radioactivity in animals were expressed as the mean±S.D. Differences between uptake values in mice tumor and in the muscles were compared using Student's *t*-test. Lack of significance was considered to be $p>0.05$.

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