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Evaluation of [^{131}I]iodoerythronitroimidazole as a predictor for the radiosensitizing effect

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The aim of this study was to evaluate whether radiolabeled iodoerythronitroimidazole (IETNIM) could predict the radiosensitization effect on tumors. Tumor-bearing mice were irradiated at a dose of 25, 31 and 37 Gy after the injection of IETNIM. They were also exposed to 37 Gy radiation at 35, 70, 140 and 240 min after the i.p. injection of IETNIM. After the irradiation, tumor growth assays were conducted and the effect of IETNIM as a radiosensitizer was estimated as enhancement factor (EF). Tumor uptake was measured at 35, 70, 140 and 240 min after i.p. injection of [^{131}I]IETNIM, which were the same intervals used in the radiosensitization study. EF of IETNIM in mice treated with 25, 30 and 37 Gy irradiation was 0.72, 0.98 and 1.28, respectively. EF of IETNIM in mice irradiated at 35, 70, 140 and 240 min after the injection was 1.50, 1.69, 1.46 and 1.08, which corresponded to the tumor uptake and blood clearance of [^{131}I]IETNIM. [^{131}I]IETNIM may be a suitable radiopharmaceutical to predict the radiosensitization effect of misonidazole analogs on tumors.

Key words: Hypoxic tumor cell, iodoerythronitroimidazole, misonidazole, radiosensitizer.

Introduction

Increasing amounts of oxygen are needed in order to keep the increasing number of tumor cells in tumors alive. Tumors produce angiogenic factors to stimulate growth of the new vessels to respond to the increasing oxygen demand, but often tumor blood flow cannot supply sufficient oxygen to the tumor. This results in low oxygen tension in tumor cells (tumor hypoxia). Under these circumstances, blood flow measurement in tumors does not adequately characterize the tumor condition. Since hypoxic tumor tissue is associated with increased resistance to radiotherapy or chemotherapy,[1,2] development of a method for measuring tumor hypoxia would be important to make an optimal decision of cancer treatment.[3]

Measurements of tumor hypoxia using an oxygen electrode,[4] the nuclear magnetic resonance (NMR) technique with perfluorocarbon (PFC)[5,6] or the nuclear medicine technique of measuring radiolabeled bioreductive drug into hypoxic tissue[3] have been proposed. *In vivo* demonstration of hypoxia by an oxygen electrode is limited in its clinical applicability due to its invasiveness. Although measurement by the NMR-based technique is non-invasive, its drawback is low temporal resolution and the need to administer large amounts of PFC that have a long half-life *in vivo*. The technique using radiolabeled bioreductive tracers may be the most realistic and desirable way of measurement.

Since nitroimidazole has a unique behavior in a low oxygen environment, many nitroimidazole analogs have been synthesized as hypoxic cell radiosensitizers.[7–9] The notion that these radiolabeled compounds might be applied to visualize hypoxic tissue *in vivo* has been discussed.[10] Misonidazole was the first of the nitroimidazoles to enter clinical trials.[11] However, few clinical trials showed the advantage of the use of misonidazole due to its side effects. For instance, peripheral neuropathy was observed at the dose level comparable to the degree of radiosensitization. In addition, the solubility at the dose required for radiosensitization was poor. Therefore, to reduce neurotoxicity and to improve solubility, it is advantageous to develop a more hydrophilic agent than misonidazole. We have previously developed ^{18}F-labeled fluoroerythromisonidazole (FETNIM) which is more hydrophilic than fluoromisonidazole (FMISO).[12]

The analogs of misonidazole labeled with ^{18}F were

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shown to selectively bind to hypoxic tumor cells.[12,13] However, these compounds require positron emission tomography (PET) and/or cyclotron facilities, which preclude their routine clinical use. By replacing ^{18}F with ^{131}I, we developed the synthesis of [^{131}I]iodoerythroimidazole (IETNIM), whose precursor is easier to prepare than that of iodomisonidazole (IMISO).[14] An additional advantage is that of using a conventional γ-camera.

The aim of this study was to evaluate whether [^{131}I]IETNIM is a suitable radiopharmaceutical to predict the radiosensitization effect of misonidazole analogs on tumors.

Materials and methods

Mice and tumors

C3Hf/Kam female and male mice (25–40 g), bred and maintained in the pathogen-free mouse colony in the Department of Experimental Radiotherapy, The University of Texas MD Anderson Cancer Center, were used.[15] They were 3–4 months old at the beginning of the experiments and were housed four to five per cage. The tumors used in this study were fourth generation isotransplants of non-immunogenic mammary carcinoma, designated MCA-4. Tumors were generated in the muscle of the right thigh of the mice by the innoculation of 5×10^5 viable tumor cells. Viability of these cells was confirmed by the Trypan blue exclusion test and phase microscopy.

Synthesis of IETNIM

IETNIM was prepared using the tosyl precursors shown in Figure 1. Briefly, a mixture of 1,4-ditosyl-2,3-isopropylidene-D-threitol (ET) (4.7 g, 10 mmol), 2-nitroimidazole (1.0 g, 9.0 mmol) and cesium carbonate (2.9 g, 9.0 mmol) in 20 ml of dimethylformamide (DMF) was heated at 60°C for 1 h. After standard extraction, a white solid (2.7 g, 6.3 mmol, 70% yield) was separated. Then, 1.0 g (2.5 mmol) of the tosyl precursor was dissolved in 5 ml acetone and stirred at 80°C in the presence of NaI (0.75 g, 5.0 mmol).[14] After 4 h the solvent was removed and the residue was taken up in ethyl acetate. The excess NaI was filtered and the solvent was removed to afford the desired compound (0.85 g, 2.3 mmol, 90% yield). The structures were determined by ^{1}H- and ^{13}C-NMR spectroscopy.[14]

Irradiation

The MCA-4 tumor-bearing mice were immobilized on a jig and tumors of the right thigh were locally irradiated with single doses of γ-radiation using a dual-source ^{137}Cs irradiator. The radiation field was 3 cm in diameter and the dose rate ranged from 6.74 to 6.69 Gy/min. The mice were irradiated without anesthesia while breathing air at atmospheric pressure.[15]

Assessment of the effect of IETNIM on radiation-induced regrowth delay

In Experiment 1, 39 tumor-bearing female mice were divided into eight groups (Table 1), each consisting of four to five mice. IETNIM was sonicated with a mixture solution (1 ml DMSO, 4 ml NaOH, 5 ml HCl, 1.5 ml $NaOCO_3$ and 2 ml ethanol) to obtain the concentration of 75 mg/ml solution. The IETNIM solution was injected i.p. at a dose of 0.5 mg/g body weight. The body weight of mice ranged from 20 to

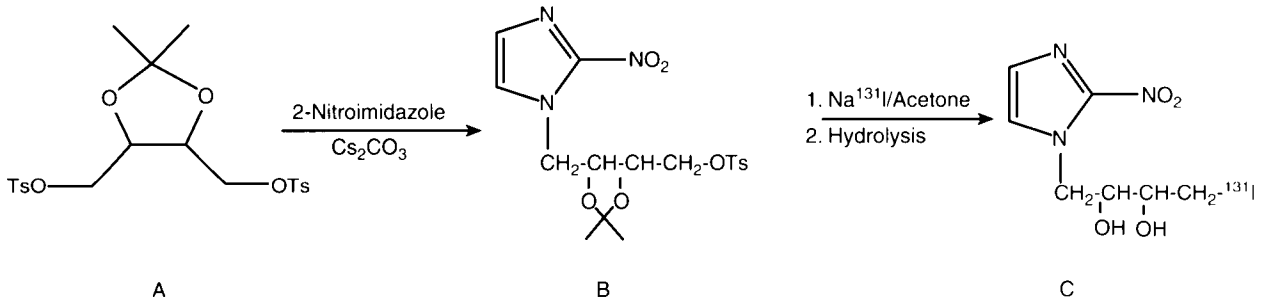

Figure 1. Synthesis of [^{131}I]Iodoerythronitroimidazole. (A) 1,4-Ditosyl 2,3,-isopylidene-threitol. (B) Tosyl erythronitroimidazole (tosyl ETNIM). (C) [^{131}I]Iodoerythronitroimidazole (IETNIM).

Table 1. Effect of IETNIM on radioresponse of MCA-4 tumor: effect of radiation dose

Group	Tumor growth[a] (days)	Absolute growth delay (days)[b]	Group	Tumor growth (days)	Statistics[c]	Normalized growth delay (days)[d]	EF[e]
Control (n 5)	6.6 ± 1.9[f]		IETNIM (n 5)	13.1 ± 3.2	p 0.02		
25 Gy (n 5)	27.8 ± 7.7	21.2	IETNIM + 25 Gy (n 5)	28.5 ± 3.3	NS	15.4	0.72
31 Gy (n 4)	29.7 ± 8.2	23.1	IETNIM + 31 Gy (n 5)	36.7 ± 8.9	NS	23.6	0.98
37 Gy (n 5)	35.9 ± 4.1	29.3	IETNIM + 37 Gy (n 5)	50.7 ± 13.0	p 0.05	37.6	1.28

[a]Time in days that tumors required to grow from 8 to 12 mm in diameter.
[b]Time in days of tumor growth in mice treated with radiation minus the time in days of tumor growth in mice of the control group.
[c]The difference in mean tumor growth (days) between mice treated with radiation alone and those with IETNIM, and the radiation at the same dose was evaluated for statistical significance using the non-parametric Mann Whitney test. Tumor growth delay in mice treated with IETNIM alone was compared with that in mice with no treatment (control group).
[d]Time in days of tumor growth in mice treated with radiation and IETNIM minus the time in days of tumor growth in mice treated with IETNIM alone.
[e]Enhancement ratio was defined as the ratio of normalized growth delay in mice treated with radiation and IETNIM over absolute growth delay in mice treated with radiation alone.
[f]Data represents mean ± SD.

26 g. Tumor growth assays were conducted by measuring three orthogonal tumor diameters with vernier calipers every day or every second day. When tumors grew to 8 mm in average diameter, they were exposed to 25, 31 or 37 Gy radiation. Tumors in mice treated with IETNIM were irradiated with the same doses at 35 min after injection. After treatment, tumor growth was followed until average tumors reached at least 12 mm in diameter.

In Experiment 2, 36 tumor-bearing male mice were divided into eight groups of five to six mice each. In this experiment, 20 mg of IETNIM was sonicated with a mixture solution (0.2 ml absolute ethanol, 0.2 ml cremophor and water 0.6 ml). The IETNIM solution was then injected i.p. at a dose of 0.5 mg/g body weight. Body weight ranged from 25 to 36 g. When tumors grew to 8 mm in diameter, they were exposed to 37 Gy radiation at 35, 70, 140 and 240 min after injection of IETNIM. The radiation dose of 37 Gy was selected on the basis of results in Experiment 1 since only a group of MCA-4 tumor bearing mice treated with 37 Gy irradiation and IETNIM showed the statistically significant radiosensitizing effect. Tumor growth assays were conducted in the same way as in Experiment 1.

In both experiments, the effect on tumor regrowth was expressed as absolute or normalized growth delay. Absolute growth delay is defined as the time in days for tumors treated with IETNIM or radiation to grow from 8 to 12 mm minus the time in days for tumors in the untreated control group to grow from 8 to 12 mm in diameter. Normalized growth delay was defined as the time for tumors in groups treated with a combination of IETNIM and radiation to grow from 8 to 12 mm minus the time of absolute growth delay in groups treated with IETNIM alone. Finally, the effect of IETNIM as a radiosensitizer was estimated as an enhancement factor (EF), which was defined as the ratio of average normalized growth delay in mice treated by the combination of IETNIM and radiation over the average absolute growth delay in mice treated with radiation alone.[15,17]

Synthesis of [^{131}I]IETNIM

An aliquot of 5 mg of tosyl precursor, tosyl erythro-nitroimidazole (TsETNIM), was dissolved in 0.5 ml acetonitrile and 50.7 MBq (1.37 mCi) of $Na^{131}I$ in 0.04 ml 1 N NaOH (Dupon NEN, Boston, MA) was added (Figure 1). The reaction mixture was heated for 1 h at 90°C. The solvent was evaporated under nitrogen, and 2 ml of chloroform and 1 ml of 5% sodium thiosulfate ($Na_2S_2O_3$) were added. Free iodine in the $Na_2S_2O_3$ layer was removed by a syringe with a 20 G needle and the remained chloroform layer was dried under nitrogen gas. Hydrolysis took place with hydrochloride (2 N, 1 ml) at 100°C for 5 min. The pH value of the final product was adjusted by adding 0.8 ml of NaOH (2 N) and 0.5 ml of $NaHCO_3$ (1 N).[14] Final products have a 0.02 retarded factor (RF) value, 99% radiochemical purity

and 0.5 Ci/μmol of specific activity. Finally, 14.8 MBq (29%) of [^{131}I]IETNIM was obtained.

Assessment of tumor uptake of [^{131}I]IETNIM

In order to evaluate the relationship between tumor uptake of [^{131}I]IETNIM and the effect of IETNIM on radiation-induced regrowth delay, 74 kBq (2 μCi) of [^{131}I]IETNIM was injected i.p., and then weight and radioactivity in tumors, muscles and blood were measured at 35, 70, 140 and 240 min after the injection, which were the same intervals between the injection of IETNIM and radiation in Experiment 2 for assessment of the effect of IETNIM on radiosensitization in MCA-4 tumors. Five mice were sacrificed at each time. Percentage of injected dose per gram tissue (%ID/g) was calculated as an index of [^{131}I]IETNIM uptake and then compared with the enhancement factor of IETNIM.

Effect of misonidazole on tissue uptake of [^{131}I]IETNIM

To assess whether the mechanism of tumor uptake of [^{131}I]IETNIM is similar to that of misonidazole, a blocking study was conducted. Ten mice were divided into two groups—one for baseline and the other one to be treated with misonidazole. In the baseline group, 74 kBq (2 μCi) of [^{131}I]IETNIM was injected i.v. via the tail vein and the mice were sacrificed 1 h after the injection. Tissue weight and radioactivity in brain, thyroid, lung, heart, liver, spleen, kidney, intestines, muscle and blood were measured, and the percentage of injected dose per gram tissue weight was estimated in each tissue. In the other group, misonidazole was dissolved in Ringer's solution (20 mg/ml) and injected i.v. at a dose of 0.2 mg/g body weight. [^{131}I]IETNIM was injected i.v. via the tail vein 30 min after the administration of misonidazole. Tissue weight and radioactivity were measured 1 h after the injection of [^{131}I]IETNIM, and compared with the indicators in the baseline group.

Statistical Analysis

The non-parametric Mann-Whitney *U*-test was used to analyze data. Two-tailed $p < 0.05$ was considered as a statistically significant difference.

Results

Effect of IETNIM on radiation-induced regrowth delay

Experiment 1. Data is summarized in Figure 2 and Table 1. In this study, IETNIM itself delayed tumor growth compared to that in untreated control mice. Radiation induced dose-dependent regrowth delay for the doses used in the present study (25–37 Gy). Only in the group of MCA-4 tumor-bearing mice treated with 37 Gy irradiation and IETNIM was there a significant sensitizing effect, with an EF of 1.28. Tumors in mice treated with 25 or 30 Gy plus IETNIM were not sensitized (EF = 0.72 and 0.98, respectively).

Experiment 2. Data is shown in Table 2. In this experiment, IETNIM itself did not delay the tumor growth compared with that in the untreated control group. The effects of radiosensitization were observed in groups of mice injected with IETNIM 35, 70 and 140 min prior to the irradiation but not in

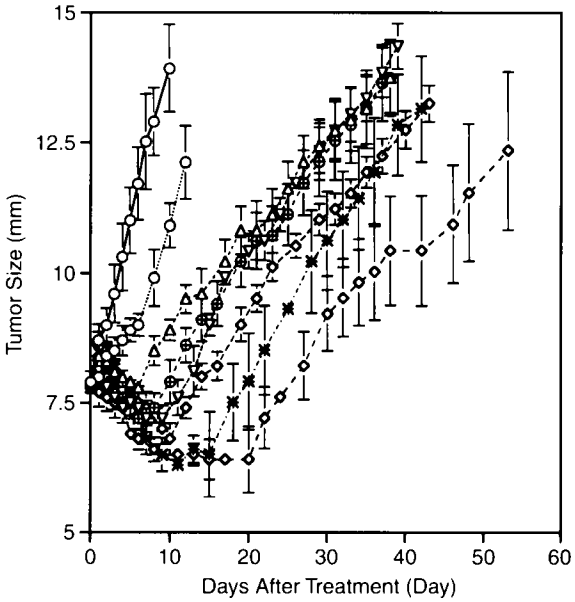

Figure 2. Effect of the combination of IETNIM and radiation on regrowth in MCA-4 mouse tumors. Mice were untreated (○) or treated with IETNIM alone at 0.5 mg/g body weight (●), with 25 Gy alone (Δ), 31 Gy alone (□), 37 Gy alone (▲) or with IETNIM (0.5 mg/g) administered 35 min prior to 25 Gy (▽), 31 Gy (⋆) and 37 Gy (◆) irradiation. Each data represents the mean diameter (mm) ± SEM. Only mice treated with IETNIM and 37 Gy radiation (◆) showed the effect of radiosensitization.

Table 2. Effect of IETNIM on radioresponse of MCA-4 tumor: effect of injection time

Group	Tumor growth[a] (days)	Absolute growth delay (days)[b]	Group	Tumor growth (days)	Statistics[c]	Normalized growth delay (days)[d]	EF[e]
Control ($n = 5$)	9.3 ± 1.9[f]		IETNIM ($n = 6$)	10.2 ± 3.6	NS		
37 Gy ($n = 5$)	44.7 ± 13.2	35.4	IETNIM 35 min ($n = 5$)	63.2 ± 4.0	$p < 0.05$	53.0	1.50
			IETNIM 70 min ($n = 5$)	70.3 ± 7.8	$p < 0.02$	60.1	1.69
			IETNIM 140 min ($n = 5$)	62.0 ± 11.8	$p < 0.05$	51.8	1.46
			IETNIM 240 min ($n = 5$)	48.3 ± 24.6	NS	38.1	1.08

[a]Time in days that tumors required to grow from 8 to 12 mm in diameter.
[b]Time in days of tumor growth in mice treated with radiation minus the time in days of tumor growth in mice of the control group.
[c]The difference in mean tumor growth (days) between mice treated with the combination of IETNIM and radiation and those with radiattion alone was evaluated for statistical significance using the non-parametric Mann Whitney test. Tumor growth in mice treated with IETNIM alone was compared with that of control group.
[d]Time in days of tumor growth in mice treated with radiation and IETNIM minus the time in days of tumor growth in mice treated with IETNIM alone.
[e]Enhancement ratio was defined as the ratio of normalized growth delay in mice treated with radiation and IETNIM over absolute growth delay in mice treated with radiation alone.
[f]Data represents mean value ± SD.

the group of mice injected with IETNIM 240 min prior to the irradiation.

Assessment of tumor uptake of [^{131}I]IETNIM

The mean ± SD of tumor diameter in this experiment was 8.2 ± 1.2 mm. The mean tumor uptake of [^{131}I]IETNIM declined as a function of time (Figure 3), and the tumor uptake at 240 min after the injection of [^{131}I]IETNIM (mean ± SEM; 1.95 ± 0.34 %ID/g) was significantly lower than that at 35, 70 and 140 min (4.11 ± 0.66, 3.95 ± 0.24, 3.51 ± 0.17 %ID/g, respectively) ($p < 0.01$). The radioactivity in blood at 240 min postinjection (4.47 ± 0.53 %ID/g) was also significantly lower than that at 35, 70 and 140 min postinjection of [^{131}I]IETNIM (7.55 ± 0.20, 8.24 ± 1.00 and 7.22 ± 0.27, respectively) ($p < 0.01$). The average muscle uptake of [^{131}I]IETNIM at 35, 70, 140 and 240 min postinjection ranged from 0.53 to 1.22 %ID/g, values which were significantly lower than tumor uptakes at each time ($p < 0.01$).

Effect of misonidazole on tissue uptake of [^{131}I]IETNIM

The tissue distribution of [^{131}I]IETNIM in the MCA-4 tumor-bearing mice in the group at baseline and that pretreated with misonidazole is shown in Table 3. The significant reduction of [^{131}I]IETNIM by the pretreatment with misonidazole was observed in tumor, intestines and liver. In this tumor model, 34% reduction of [^{131}I]IETNIM uptake was shown by pretreatment with misonidazole. Tumor to muscle ratio was 1.711 ± 0.187 %ID/g after the injection of misonidazole, which was also significantly ($p < 0.005$) lower than that obtained in the baseline group.

Discussion

The present study demonstrated that IETNIM had a radiosensitization effect on MCA-4 tumors. The EF ranged from 1.28 to 1.69 in the tumor growth delay assay (Tables 1 and 2). The degree of tumor radiosensitization by IETNIM was radiation dose-dependent within the range of dose used in Experiment 1 (Table 1). Two possibilities may account for the radiation dose-related tumor regrowth delay. One possibility is the tumor bed effect, which means that tumors in tissues damaged by radiation grow slower.[15,17] The other one is that IETNIM enhanced the radiosensitivity of hypoxic cells because the tumor response with high radiation doses is dominated by the response of hypoxic cells.[15] The median pO_2 value for untreated 8 mm MCA-4 tumors was 6.2 mm Hg (ranging between 0 and 32 mm Hg),

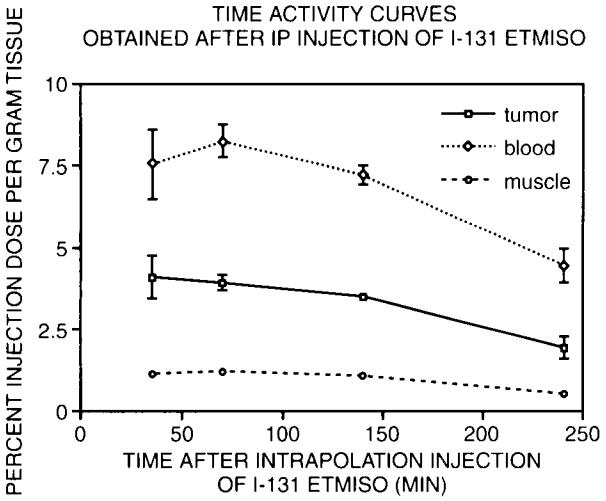

Figure 3. Tumor, blood and muscle uptake of [^{131}I]IETNIM after i.p. injection. Each point is given as the mean ± SEM. The mean tumor uptake of [^{131}I]IETNIM declines as a function of time and the tumor uptake at 240 min after the injection of [^{131}I]IETNIM (mean ± SEM; 1.95 ± 0.34 %ID/g) was significantly lower than that at 35, 70 and 140 min. The peak radioactivity in blood (mean ± SEM; 8.24 ± 0.50 %ID/g) was observed at 70 min postinjection and radioactivity in blood at 240 min postinjection (4.47 ± 0.53 %ID/g) was significantly lower than that at 35, 70 and 140 min postinjection of [^{131}I]IETNIM.

which was measured using a Polarographic pO_2 Histograph (Eppendorf, Hamburg, Germany).[18]

The differences in tumor growth with IETNIM alone in Experiments 1 and 2 may be due to the different vehicle used for injection, but this is not conclusive. This compound was dissolved in a mixture solution (1 ml DMSO, 4 ml NaOH, 5 ml HCl, 1.5 ml $NaOCO_3$ and 2 ml ethanol) and approximately 30% of mice which received IETNIM died during Experiment 1. After we changed components of the mixture solution in Experiment 2, none of the mice which received the same dose of IETNIM died. The ethanol vehicle and DMSO could compromise renal glomeruli, perphaps by dissolving membranes and resulting in blood in the urine. This situation could produce morbidity and death in Experiment 1. Although there are no explanations for this inconsistent result at present, one possibility is that mice in Experiment 1 might have been under poor nutrition. In the remaining 70% of mice a delay in tumor growth can be attributed to inadequate nutrition. However, the data of tumor growth delay assay in Experiment 1 is reliable because the normalized tumor growth delay was used as a quantitative index to evaluate the radiosensitization by IETNIM.

In this study, MCA-4 tumor-bearing mice irradiated at 30–140 min after the injection showed the radiosensitization effect by IETNIM, which was related to the tumor uptake and blood clearance of [^{131}I]IETNIM (Table 2 and Figure 3). It has been reported that despite the complexity of the intracellular distribution of radiosenstizers, variations in radiosensitization levels were well correlated with measurements of average intracellular concentration.[19] The nitroimidazole concentration at the time of irradiation is not the only factor determining sensitization. Preradiation depletion of thiols by exposure to drugs also may be a factor. From these findings, measurements of tumor uptake with [^{131}I]IETNIM may be useful in deciding the suitable

Table 3. Effect of misonidazole on biodistribution in mice[a] of [^{131}I]IETNIM[b]

Organ	Treated with misonidazole[c]	Baseline	Ratio	Statistics
Brain	0.825 ± 0.221[d]	0.573 ± 0.115	1.44	NS
Thyroid	31.100 ± 7.356	28.232 ± 11.809	1.10	NS
Lung	1.968 ± 0.133	2.243 ± 0.372	0.87	NS
Heart	1.441 ± 0.158	1.398 ± 0.352	1.03	NS
Liver	1.844 ± 0.326	2.314 ± 0.190	0.80	$p < 0.05$
Spleen	1.570 ± 0.340	1.645 ± 0.256	0.95	NS
Kidney	4.927 ± 0.491	4.274 ± 1.068	1.15	NS
Intestines	1.551 ± 0.265	2.179 ± 0.433	0.71	$p < 0.05$
Muscle	1.027 ± 0.143	0.877 ± 0.103	1.17	NS
Blood	2.570 ± 1.098	3.017 ± 1.299	0.85	NS
Tumor	1.753 ± 0.277	2.514 ± 0.553	0.66	$p < 0.05$
Tumor/muscle	1.711 ± 0.172	2.950 ± 0.981	0.58	$p < 0.005$
Tumor/blood	0.738 ± 0.187	0.878 ± 0.138	0.84	NS

[a]C3Hf/Kam male mice (3 months old).
[b][^{131}I]IETNIM was injected i.v. and mice were sacrificed at 1 h postinjection.
[c]Non-radiolabeled misonidazole was injected 30 min prior to the injection of [^{131}I]IETNIM.
[d]Data represents mean value and standard deviation ($n = 5$).

radiation therapy schedule using radiosensitisers to predict the radiosensitization level by misonidazole analogs in each patient.

Tumor, liver and intestine uptakes of [^{131}I]IETNIM were suppressed by misonidazole in our study (Table 3). When misonidazole is used at a pharmacologic dose, it is metabolized in the liver, and saturation of metabolic and excretory mechanism may occur.[3] Misonidazole enters cells by passive diffusion and is reduced intracellularly in all cells with viable nitroreductase enzymatic processes, but only in hypoxic cells do they undergo further reduction and remain in the cells. As mentioned above, MCA-4 tumor cells used in this study are hypoxic (median pO_2 in the tumor was 6 mm Hg) and untreated MCA-4 tumor (8 mm in diameter) contained 32% hypoxic cells.[18] From these findings, we propose that the trapping mechanism of [^{131}I]IETNIM in the tumor may be similar to that of misonidazole. In other words, [^{131}I]IETNIM may accumulate in hypoxic tumor cells in the same way as misonidazole. There were presumably some hypoxic cells in the intestines.

Recently, ^{99m}Tc- or ^{123}I-labeled nitroimidazoles have been developed.[20–25] From the view point of the characteristics of the radionuclide, these tracers are more suitable to obtain a high quality image with a γ-camera. We used the ^{131}I for labeling IETNIM in this study and it is easy to substitute ^{123}I for ^{131}I to produce [^{123}I]IETNIM, which is more desirable for superior imaging. We should improve the radiochemical yield to produce IETNIM with ^{123}I because a 20–30% radiochemical yield is not practical. Tumor/blood and tumor/muscle ratios are the important parameters which determine image quality *in vivo*. The tumor/blood ratio was less than 1.0 and the tumor/muscle ratio was modest in comparison with [^{18}F]ETNIM.[12] It may be caused by the high partition efficient and the *in vivo* instability based on the primary carbon substituted iodine as in IETNIM.

In summary, IETNIM acts as a radiosensitizer for MCA-4 tumor and the mechanism of tumor uptake of [^{131}I]IETNIM is similar to that of the well known radiosensitizer, misonidazole. [^{131}I]IETNIM may be a suitable radiopharmaceutical to predict the radiosensitization effect of misonidazole analogs on tumors.

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