Radioiodinated 1-(5-Iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole (Iodoazomycin Arabinoside: IAZA): A Novel Marker of Tissue Hypoxia

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1-(5-Iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole (IAZA) has been synthesised and labeled with ^{125}I. Radioiodinated IAZA was shown to undergo hypoxia-dependent binding in EMT-6 cells in vitro and to have an initial binding rate of 284 pmole/10^6 cells/hr at a substrate concentration of 30 μM. This binding rate is more than three times that of the reference compound, misonidazole (89 pmole/10^6 cells/hr). The elevated binding rate was accompanied by in vitro cytotoxicity 30–40 times greater than that observed for misonidazole. Whole-body elimination and biodistribution studies in BALB/c mice bearing implanted, subcutaneous EMT-6 tumors showed a rapid excretion (>98% in 24 hr) with moderate tissue levels which, in general, declined as a function of blood clearance. Tumor-to-blood ratios of 4.6 (4 hr) and 8.7 (8 hr), with respective tumor uptake values of 2.08% and 1.22% ID/g of tissue, form a rational basis for evaluation of this and related 2-nitroimidazole analogs as radiopharmaceuticals suitable for scintigraphic evaluation of tissue (tumor) hypoxia.

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Radiosensitizers are agents which enhance the lethal effects of ionizing radiation. The ideal radiosensitizer remains elusive, despite the synthesis and evaluation of a large number of electron affinic compounds. Nitroimidazoles, particularly N^1-substituted derivatives of 2-nitroimidazole (azomycin), have been among the most promising agents tested. Of the compounds examined, those that have single electron reduction potentials (E_7^1) of −380 to −400 mV and partition coefficients of 0.1–10 have shown the greatest efficiacy and lowest toxicity in vitro and in vivo (*1–4*). The E_7^1 value is important because it reflects the ability of the compound to accept electrons from the cytochrome system. This is the first step in the formation of reactive reduction products. It has also been demonstrated that the formation of tissue adducts with reactive reduction intermediates of these compounds results in their selective metabolic trapping by hypoxic cells (*5–7*). The partition coefficient governs the ability of the radiosensitizer to cross biological membranes and can influence its movement to hypoxic tissues.

The degree of tumor hypoxia is a vital consideration in the design of a radiotherapy regimen and the presence of hypoxic cells in tumors is regarded as a major determinant in the failure of radiotherapy to completely eradicate tumor tissue. The selective hypoxia-dependent binding of gamma-emitting nitroimidazole derivatives could therefore make them prospective diagnostic agents for the in vivo scintigraphic detection and assessment of tissue hypoxia (*8*). This approach has been explored recently in the development of agents such as 4-bromomisonidazole (*9, 10*), fluoromisonidazole (*11,12*) and iodoazomycin riboside (*13,14*). Of these compounds, fluoromisonidazole has shown the most potential and as its ^{18}F analog has yielded images of ischemic myocardium (*15*) and hypoxic tumor tissue (*16*).

Several radioiodinated 2-nitroimidazole radiosensitizers have been developed in our search for a suitable imaging agent. Compounds such as 1-{2-(2-iodophenoxy)-ethyl}-2-nitroimidazole (IPENI) (*17*) and the iodohydroxyacetophenones (*18*) were found to be highly lipophilic. These compounds demonstrated in vivo stability toward deiodination but with minimal tumor uptake, high levels of protein binding in the blood and a cellular uptake apparently independent of tissue hypoxia. Radioiodinated 1-(5-iodo-5-deoxy-β-D-ribofuranosyl)-2-nitroimidazole 4 (iodoazomycin riboside: IAZR) was designed to overcome the highly lipophilic nature of these earlier radioiodinated compounds without affecting the electron affinic properties of the 2-nitroimidazole ring (*13*). This nucleoside displayed radiosensitizing properties of greater potency than misonidazole and high affinity for an equilibrative membrane transporter of nucleosides in vitro (*13*). It was

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also more toxic in vitro than misonidazole and radiochemically unstable in vivo as evidenced by tissue distribution of activity characteristic of radioiodide. Deiodination was believed to occur following enzymatic cleavage of the glycosidic bond rather than by directly from the intact ^{125}I-IAZR (*14*). A number of arabinosyl sugar nucleosides have been shown to be more stable than their ribosyl counterparts in in vivo and in in vitro studies (*19*). Therefore, 1-(5-iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole 3 (iodoazomycin arabinoside: IAZA) has now been synthesised to exploit the adduct-forming properties displayed by IAZR combined with the anticipated in vivo stability of the arabinosyl-N^1-β-glycosidic bond against enzymatic cleavage.

MATERIALS AND METHODS

All chemicals were reagent grade. Na^{125}I, as a solution in N/10 NaOH, was purchased from the Edmonton Radiopharmacy Center. Solvents were distilled before use, and where anhydrous solvents were required, they were dried by standard methods. TLC was carried out with Whatman MK6F microplates, using chloroform:methanol = 9:1 (v/v) as a developing solvent. Silica gel (60-100 mesh) was used for column chromatography. HPLC was performed with a Waters reverse-phase C-18 radial compression column eluted with methanol:water = 40:60 (v/v). A NaI(Tl) scintillation detector was used for on-line determination of radioactivity in column effluent (radio-HPLC). Melting points were determined with a Büchi melting point apparatus, and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AM-300 spectrometer, low-resolution mass spectra were determined on a Hewlett-Packard 5995 mass spectrometer (DIP, E_V = 70 keV). High-resolution mass spectra were measured using an AEI MS-50 mass spectrometer and were used in lieu of elemental analysis to determine the molecular formulae. Details of the synthesis are reported in the Appendix.

In Vivo Biodistribution of ^{125}I-IAZA

Male BALB/c mice (20–25 g) were inoculated subcutaneously in the left flank with a suspension of murine EMT-6 cells (10^5 cells in 0.1 ml) (*14*). After 12–14 days, when the tumors reached the desired size (8–10 mm diameter), each mouse received a single intravenous injection of ^{125}I-IAZA (59 kBq in 0.1 ml). Animals (six per time interval) were exsanguinated by cardiac puncture immediately following asphyxiation in CO_2, at intervals of 15 min, 30 min, and 1, 2, 4, 8, 12, and 24 hr after injection of ^{125}I-IAZA. Heart, lung. liver, spleen, muscle, bone, thyroid, kidney, stomach, small intestine, tail, tumor and skin were removed upon necropsy, weighed and analyzed for ^{125}I using a Beckman Model 8000 gamma scintillation counter. The remaining carcass mass was also weighed and radioassayed. Summed activities for all dissected tissues and residual carcass were used to determine the whole-body activity.

In Vitro Cytotoxicity

The cytotoxicity of IAZA toward EMT-6 cells in culture was determined by exposing stirred cell suspensions to various drug concentrations under both aerobic and hypoxic conditions. Colony forming ability of cell aliquots removed at various incubation times was then determined as described elsewhere (*13*). Exposure times of up to 6 hr, for IAZA concentrations ranging from 0.1 to 1.0 m*M*, were used.

Initial Binding Rates

Initial binding rates were measured at four concentrations of ^{125}I-IAZA (3, 10, 30, and 100 μ*M*), using normally oxygenated and hypoxic EMT-6 cell cultures (*13*). Aliquots of the exposed cells were removed at set time intervals, treated with trichloroacetic acid to affect precipitation of the macromolecular fraction and filtered. Activity trapped on the filters was determined by gamma scintillation counting. Control studies were performed at the same concentration with misonidazole.

Partition Coefficients

Partition coefficients of unlabeled compounds were determined by HPLC quantitation after distribution between 1-octanol and 0.05 *M* phosphate buffer (pH 7.4).

In Vitro Phosphorylase Activity

The in vitro phosphorolysis of the sugar-coupled nitroimidazoles was studied by incubating the unlabeled test compounds (250 μ*M* concentration) with commercial thymidine phosphorylase from E. Coli (Sigma Chemical Co.). Deoxyuridine and deoxythymidine were tested in parallel as controls and the degree of enzymatic cleavage of the compounds was determined by HPLC analysis of the reaction products (ultraviolet absorption at 350 nm) after various incubation times. The procedure is described in detail elsewhere (*20*).

RESULTS AND DISCUSSION

Table 1 presents the tissue distribution data for six mice at each of seven time intervals after injection of ^{125}I-IAZA. With the exception of the liver and kidney, the initial distribution of ^{125}I-IAZA appears to reflect blood perfusion in each organ or tissue. There is no indication of selective uptake or binding of radioactivity in the normal (non-tumor) tissues studied. This is reflected in the rapid decline in the blood activity-time curve in the distribution phase and the similarities between blood clearance and whole-body elimination at later stages, as shown in Figure 1. Early hepatic uptake was still evident after 1 hr (Table 1), but the relatively low level of ^{125}I in the intestinal tract at longer intervals does not support a mechanism of hepatobiliary excretion. Early renal activity reflects urinary elimination of the water-soluble nucleoside analog. Although no attempt was made to measure urinary radioactivity, balance studies do indicate a gradual whole-body elimination which accounts for about 98% of the dose over 24 hr (Fig. 1). Gradual deiodination of ^{125}I-IAZA in vivo is suggested by the increase in activity in the thyroid at later time periods.

Whole-body elimination half-lives are estimated to be 0.87 hr and 7.29 hr, based on the 1–24-hr elimination data analyzed as a biexponential function. The blood clearance data form a more complex curve which is not resolved by simple exponential analysis but which is characterized by an initial very rapid distribution phase and a much slower clearance during the 12–24-hr period. The concentration of radioactivity in tumor reaches a maximum of 3.5% of the ID/g of tissue at 0.5 hr, after which the level declines slowly to 1.2% at 8 hr. The decline in tumor activity probably represents for the most part clearance of un-

TABLE 1
Percent of Injected Dose per Gram of Tissue in BALB/c Mice Bearing Subcutaneous EMT-6 Tumors After Intravenous Injection of ^{125}I-IAZA

Organ	Time (hr)						
	0.25	0.50	1	2	4	8	24
Blood	2.63 ± 0.30	2.17 ± 0.42	1.84 ± 0.67	0.91 ± 0.20	0.45 ± 0.32	0.14 ± 0.03	0.037 ± 0.007
Tumor	2.55 ± 0.28 (0.97)	3.48 ± 0.85 (1.60)	2.70 ± 0.07 (1.46)	2.55 ± 1.56 (2.80)	2.08 ± 1.24 (4.62)	1.22 ± 0.07 (8.71)	0.206 ± 0.047 (5.56)
Kidney	6.56 ± 0.63 (2.49)	5.67 ± 0.95 (2.61)	4.38 ± 1.25 (2.38)	1.80 ± 0.61 (1.98)	0.66 ± 0.39 (1.46)	0.34 ± 0.06 (2.43)	0.52 ± 0.004 (1.41)
Liver	8.65 ± 1.02 (3.29)	7.14 ± 1.13 (3.29)	5.63 ± 2.27 (3.06)	2.18 ± 0.80 (2.39)	0.95 ± 0.58 (2.11)	0.47 ± 0.10 (3.36)	0.098 ± 0.008 (2.64)
Heart	4.69 ± 0.91 (1.78)	3.56 ± 0.66 (1.64)	2.78 ± 1.30 (1.51)	1.03 ± 0.43 (1.13)	0.32 ± 0.24 (0.71)	0.22 ± 0.05 (1.57)	0.042 ± 0.017 (1.14)
Spleen	3.57 ± 0.67 (1.36)	3.00 ± 0.62 (1.38)	2.48 ± 1.25 (1.35)	0.93 ± 0.37 (1.02)	0.35 ± 0.20 (0.78)	0.18 ± 0.04 (1.29)	0.036 ± 0.034 (0.97)
Muscle	3.09 ± 0.67 (1.17)	2.77 ± 0.54 (1.28)	2.19 ± 1.03 (1.19)	0.69 ± 0.29 (0.75)	0.24 ± 0.12 (0.53)	0.36 ± 0.31 (2.57)	0.020 ± 0.004 (0.54)
Bone	1.31 ± 0.18 (0.50)	1.37 ± 0.57 (0.63)	1.15 ± 0.52 (0.63)	0.58 ± 0.26 (0.64)	0.27 ± 0.09 (0.60)	0.08 ± 0.03 (0.57)	0.022 ± 0.03 (0.59)
Intestine	5.42 ± 1.03 (2.06)	4.44 ± 0.62 (2.04)	4.63 ± 1.92 (2.52)	1.73 ± 0.55 (1.90)	0.81 ± 0.46 (1.80)	0.77 ± 0.36 (5.50)	0.041 ± 0.016 (1.11)
Stomach	4.36 ± 0.70 (1.66)	4.75 ± 1.05 (2.19)	3.95 ± 0.93 (2.15)	3.30 ± 0.93 (3.62)	1.81 ± 1.60 (4.02)	0.87 ± 0.54 (6.21)	0.067 ± 0.014 (1.81)
Lung	4.61 ± 0.82 (1.75)	3.77 ± 0.67 (1.74)	2.99 ± 1.41 (1.63)	1.15 ± 0.38 (1.26)	0.45 ± 0.30 (1.00)	0.33 ± 0.07 (2.36)	0.037 ± 0.008 (1.00)
Carcass	2.68 ± 0.44 (1.02)	2.55 ± 0.37 (1.18)	2.23 ± 0.91 (1.21)	1.46 ± 0.29 (1.60)	0.89 ± 0.52 (1.98)	0.28 ± 0.12 (2.00)	0.038 ± 0.016 (1.03)
Thyroid	0.06 ± 0.03	0.08 ± 0.04	0.15 ± 0.10	0.37 ± 0.25	0.36 ± 0.23	0.42 ± 0.10	0.77 ± 0.22

The data are mean values for n = 6, ± standard deviation. Data in parentheses are tissue-to-blood ratios. Thyroid data are presented as %ID in the organ.

bound drug from the oxygenated portions of the tumor while residual activity represents activity trapped as cellular adducts in the hypoxic tissue. This slow decline in radioactivity is in contrast to most other tissues, which show decreasing radioactivity levels which essentially mimic declining blood radioactivity levels. The maximum tumor-to-blood ratio of 8.7 is reached at 8 hr. There is a six-fold decline in tumor radioactivity at 24 hr compared to the 8-hr levels, but this still represents a tumor-to-blood ratio in excess of 5.5 (Fig. 2).

It is believed that the uptake of IAZA into tumor tissue is due to hypoxia-dependent binding. Studies with EMT-6 tumors have shown that they have a high hypoxic fraction (0.33) (*21*). The administration of ^{14}C-misonidazole to BALB/c mice bearing EMT-6 tumors led to selective labeling of the tumor tissue. This labeling was shown to be due to adduct formation between the sensitizer and cellular macromolecules in a process which was selective to the reducing environment of the hypoxic tissues (*5*).

In vitro binding studies of ^{125}I-IAZA were carried out with hypoxic and normally oxygenated EMT-6 cells (Fig. 3). The binding of ^{125}I-IAZA was linear over a 3-hr incubation period at concentrations of 10–100 μM. It is also notable that very little uptake of activity occurs under oxic conditions with a substrate concentration of 30 μM. These results indicate hypoxia-dependent binding and are qualitatively similar to the binding behavior observed with misonidazole (*5*) and with IAZR (*13*).

The relative binding effectiveness of compounds can be compared by determining the initial binding rates, derived from the data in Figure 3 and the known specific activity of the radiolabeled test drug. This data is presented in terms of picomoles of substrate bound to 10^6 cells per hour at various concentrations. The initial binding rates for IAZA, IAZR and misonidazole are given in Figure 4. The initial binding rate for IAZA over the concentration range of 10–100 μM was comparable to that for IAZR (*13*) and was two to three times greater than misonidazole under similar conditions (Fig. 4). At 30 μM concentration, the binding rate for ^{125}I-IAZA under hypoxic conditions (280 pmole/10^6 cells/hr) was over seven times greater than the binding rate in oxygenated cells (38 pmole/10^6 cells/hr).

Figure 5 shows the surviving fraction of EMT-6 cells in culture after exposure to various concentrations of IAZA under hypoxic incubation at 37°C. These data are characteristic of hypoxic cell radiosensitizers such as misonidazole and IAZR in that they show increasing toxicity with increasing drug concentration and a relative absence of toxicity in the control experiment with an oxic environment. The time required to reduce the cell population by 90% at 1.0 mM concentration of IAZA is 1.65 hr, which is similar to IAZR (1.7 hr) and much less than misonida-

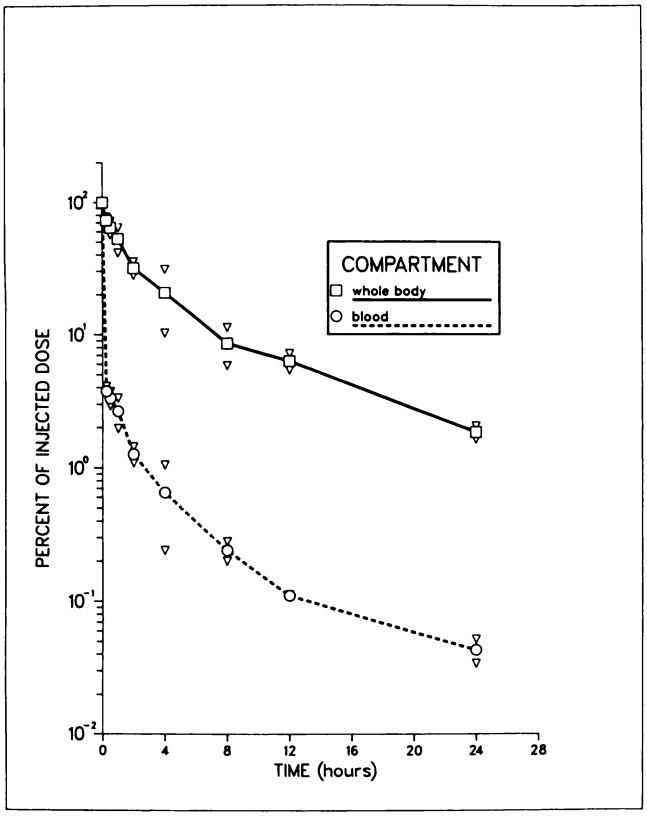


FIGURE 1. Blood clearance and whole-body elimination of radioactivity in BALB/c mice bearing subcutaneous EMT-6 tumors after intravenous injection of ^{125}I-IAZA. The Δ represents the standard deviation limits of the mean value for six animals.

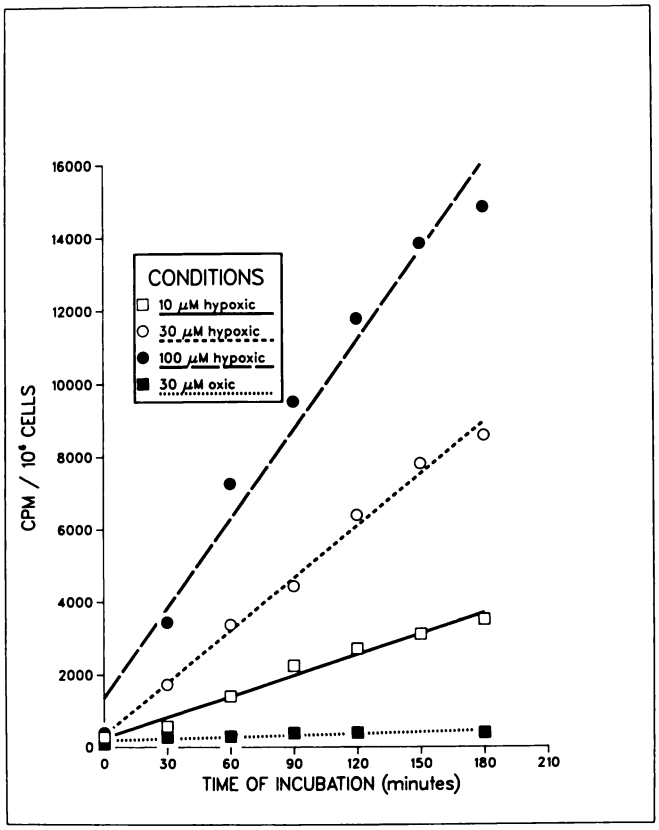


FIGURE 3. Uptake of radioactivity into the acid insoluble fraction of EMT-6 cells during incubation with various concentrations of ^{125}I-IAZA (specific activity = 0.23 GBq/mmol) under oxic and hypoxic conditions at 37°C.

zole (11 hr) (*13*). IAZA must therefore be regarded as considerably more toxic to hypoxic cells than misonidazole.

The relatively facile uptake of IAZA into most cells is demonstrated by the tissue distribution studies using radiolabeled drug. Although the compound is readily cleared from oxic tissues, there is some accumulation of radioactivity in hypoxic cells probably due to subsequent cellular reduction processes. Biologic reduction of the nitro group and the formation of toxic metabolites have been demonstrated in studies with misonidazole and other 2-nitroimidazoles. The biochemical processes responsible for the reduction of nitro heterocycles have been extensively investigated (*22*) and a wide range of biologic consequences have been identified, including cytotoxicity, chemopotentiation, radiosensitization, and cellular adduct formation (*23*). The increased toxicity of IAZA and IAZR to hypoxic cells, relative to misonidazole, may be due to the greater lipophilicity of these compounds as demonstrated by their partition coefficients (Table 2). The more lipophilic sugar coupled nitroimidazoles (IAZA and IAZR) can be expected to cross-cellular membranes more readily than misonidazole.

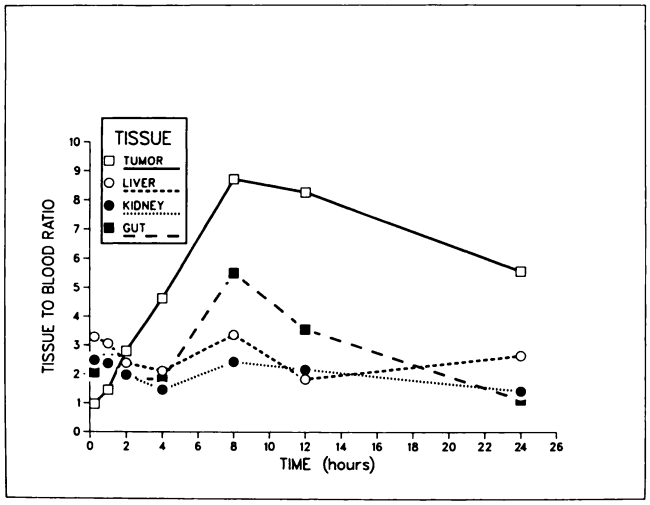


FIGURE 2. Tissue-to-blood radioactivity levels for selected tissues in BALB/c mice bearing subcutaneous EMT-6 tumors after intravenous injection of ^{125}I-IAZA.

While IAZA and IAZR have similar in vitro characteristics, they differ substantially in some in vivo studies. Tumor-to-blood ratios for IAZR reached a maximum of about 5.5 at 3 hr in the BALB/c mouse EMT-6 tumor model. The compound was extensively degraded in vivo as indicated by high levels of radioiodide in the blood plasma and urine (*14*). The ribofuranose structure of IAZR was considered to be more sensitive to phosphoro-

TABLE 2
Octanol-Water Partition Coefficient Values for 2-nitroimidazoles

Compound	p	Reference
Misonidazole	0.43	13
IAZR	2.1	13
IAZA	4.98	this work
4-Bromomisonidazole	2.87	12
Fluoromisonidazole	0.40	12
IPENI	694	17

lytic cleavage by enzymes than the arabinofuranose structure of IAZA. This was not confirmed by in vitro phosphorolysis studies in which these two compounds were incubated with bacterial phosphorylase. Both compounds were resistant to phosphorolytic cleavage of the sugar-nitroimidazole bond under conditions which lead to substantial cleavage of deoxyuridine and thymidine. The in vivo superiority of IAZA over IAZR in murine studies has not been explained, although it may be due in part to the somewhat higher lipophilicity (Table 2) and hence enhanced diffusion of IAZA across cell membranes.

It is concluded that IAZA exhibits hypoxia-dependent binding, characteristic of and somewhat greater than misonidazole. The greater cytotoxicity exhibited by IAZA is a reflection of its high initial binding rate and may indicate some chemotherapeutic potential for this type of compound in the treatment of hypoxic tumors. The increased cytotoxicity to hypoxic tissue is not significant in the proposed scintigraphic utilization of IAZA because of the small molar amounts of the radiolabeled compound (^{123}I-IAZA) that will be required. Whole-body distribution studies indicate that tumor uptake and tumor-to-blood ratios for ^{125}I-IAZA are superior to those for ^{131}I-IAZR (*14*) and other gamma-emitting radiolabeled nitroimidazoles, such as 4-bromomisonidazole (*9*) and the positron-emitting ^{18}F-fluoromisonidazole (*12*), and appear to be reflective of both high binding rates and improved radiochemical stability.

Direct comparisons between IAZA and other proposed hypoxic imaging agents is complicated by the use of a variety of test systems and time periods for the studies. Table 3 shows the measurements of tumor and blood activity at 2 hr for misonidazole and several 2-nitroimidazoles proposed as scintigraphic agents for the detection of tissue hypoxia. IAZA appears to have both good tumor uptake and blood clearance characteristics and therefore gives a relatively high tumor to blood ratio. The high tumor-to-blood ratio for IAZR at 3 hr seems to be mainly radioiodide distribution rather than hypoxic tissue binding of intact compound. The relatively slow clearance of unbound IAZA from blood and tissues in the mouse model is comparable to that observed with other 2-nitroimidazoles. The optimum time for scintigraphic imaging with radioiodinated IAZA in the BALB/c mouse model would be between 4 and 8 hr. It is likely that optimum imaging times in humans would be at even later times after drug administration. This factor would appear to limit the usefulness of short-lived radionuclides such as ^{18}F ($t_{1/2}$ =

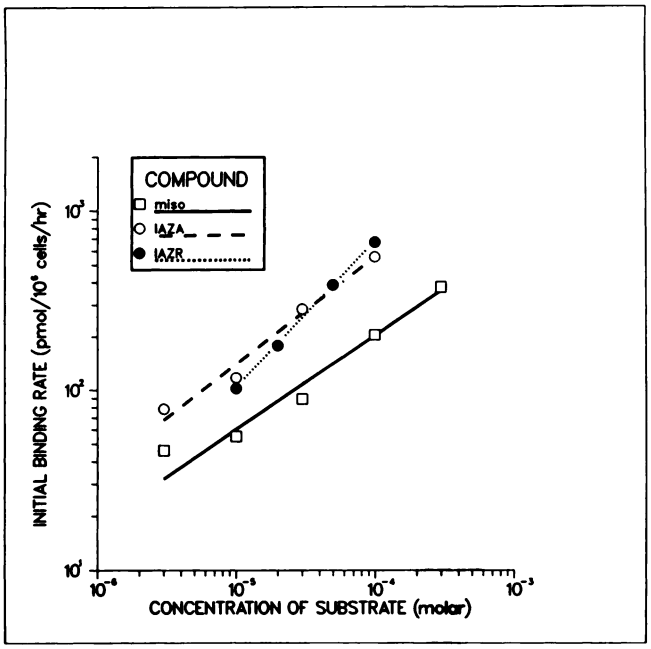


FIGURE 4. Initial binding rate of ^{125}I-IAZA to the acid insoluble fraction of EMT-6 cells under hypoxic conditions at 37°C.

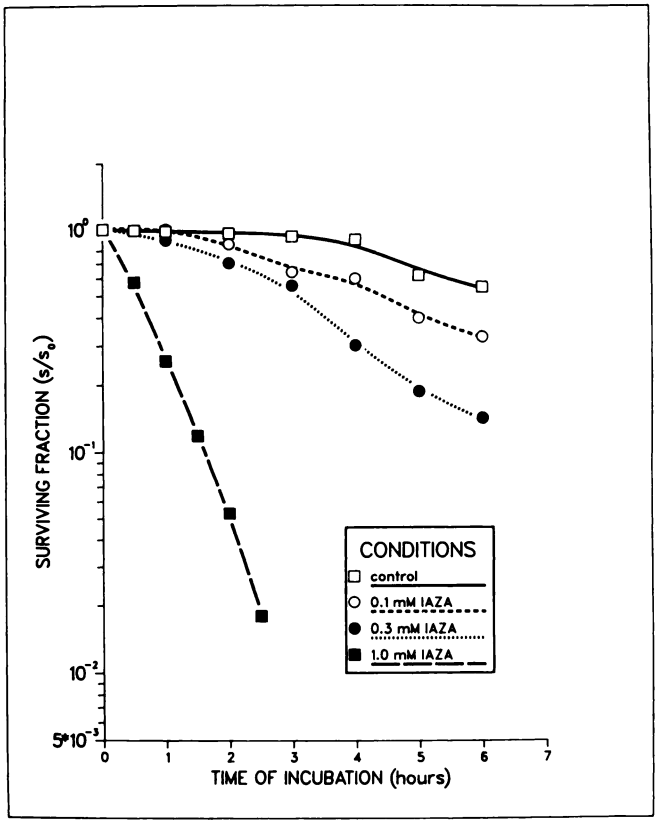


FIGURE 5. Surviving fractions EMT-6 cells incubated with various concentrations of IAZA.

TABLE 3
Tissue Uptake and Tumor-to-Blood Ratios at 2 Hours for Some 2-nitroimidazoles

Compound	Isotopic label	% ID/g		Tumor/Blood ratio	Tumor model	Reference
		Tumor	Blood			
Misonidazole	^{3}H	1.37 ± 0.45	0.73 ± 0.23	1.88	C3H mice/KHT tumor	12
IAZR	^{131}I	0.28 ± 0.15	0.19 ± 0.02	1.47	BDF_1 mice/LL tumor	14
IAZR*	^{131}I	9.83 ± 3.20	1.79 ± 0.58	5.49	BALB/c mice/EMT-6 tumor	14
IAZA	^{125}I	2.55 ± 1.56	0.91 ± 0.20	2.80	BALB/c mice/EMT-6 tumor	this work
4-Bromomisonidazole	^{82}Br	1.10 ± 0.19	2.77 ± 1.05	0.40	BALB/c mice/EMT-6 tumor	12
Fluoromisonidazole	^{3}H	1.29 ± 0.20	0.98 ± 0.07	1.31	BALB/c mice/EMT-6 tumor	12

* At 3 hr after drug administration.

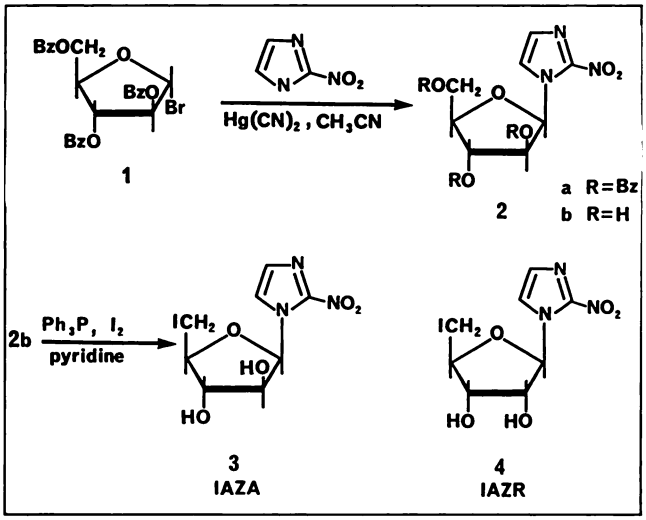
110 min), suggested as a label for fluoromisonidazole, but is not an impediment for scintigraphic studies with IAZA labeled with ^{123}I ($t_{1/2}$ = 13 hr).

These data are strongly supportive of a role for ^{123}I-IAZA in hypoxia-specific diagnostic scintigraphy, and provide a rational basis for continued evaluation of this novel radiolabeled marker of tissue hypoxia. We have initiated a clinical trial with ^{123}I-IAZA in human cancer patients and preliminary planar and SPECT scintigrams have shown elevated uptake of radioactivity within areas of some tumors believed to contain hypoxic tissue.

APPENDIX

1-(2,3,5-Tri-O-benzoyl-β-D-arabinofuranosyl)-2-nitroimidazole, 2a

The coupling procedure of Sakaguchi et al. (*24*) was modified to give a higher yield and selective formation of the β-anomer (Scheme 1). 2-Nitroimidazole (118 mg; 1.05 mmol) was added to a stirred solution of 1-bromo-2,3,5-tri-O-benzoyl-α-D-arabinofuranose **1** (500 mg; 0.95 mmol) and mercuric cyanide (600 mg; 2.04 mmol) in dry acetonitrile (50 ml). The mixture was stirred for 6 hr at room temperature, after which the solvent was removed under vacuum. The residue was dissolved in dichloromethane (200 ml) and filtered. The filtrate was washed successively with saturated aqueous sodium hydrogen carbonate solution, 30% aqueous potassium iodide, and water, then dried over anhydrous magnesium sulfate. The solvent was evaporated under vacuum and the residue applied to a silica gel column and eluted with toluene:ethyl acetate = 90:10 (v/v). The coupled product **2a** was recovered in 69% chemical yield (367 mg).



SCHEME 1. Selective formation of the β-anomer.

1-(β-D-Arabinofuranosyl)-2-nitroimidazole, 2b (AZA)

The previous product **2a** (250 mg; 0.45 mmol) was dissolved in methanolic ammonia (25 ml) and allowed to stand at 0°C for 2 days. The solvent was removed under vacuum and the residue was washed three times with chloroform. The washed residue was dissolved in methanol and the title compound (AZA) was recrystallized from this solution in 92% (101 mg) recovered yield. MP. 192–193°C (lit. 160°C (*12*)); ^{1}H NMR (CD_3OD) δ 7.65 (1H, d, J = 1.3 Hz, C_5-H); 7.14 (1H, d, J = 1.3 Hz, C_4-H); 6.44 (1H, d, J (C_1'-C_2') = 1.3 Hz, C_1'-H); 4.50 (1H, m, C_4'-H); 4.25 (1H, m, C_2'-H); 4.14 (1H, m, C_3'-H); 3.78 (2H, m, C_5'-H). ^{13}C NMR (CH_3OD) δ 145 (C_2); 128.1 (C_4); 125.2 (C_5); 97.1 (C_1'); 91.7 (C_4'); 84.0 (C_2'); 78.1 (C_3'); and 63.2 (C_5'). MS (DIP E_V = 70 eV) 245 (M^+; 5%). Exact mass 245.0467, calculated 245.0468 for $C_8H_{11}N_3O_6$.

(1-(5-Iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole, 3 (IAZA)

AZA **2b** (100 mg; 0.4 mmol) in dry pyridine (5 ml) was mixed with triphenylphosphine (212 mg; 0.8 mmol) and iodine (101 mg; 0.40 mmol), and stirred for 4 hr at 30°C. The reaction was quenched with methanol (0.5 ml), after which the mixture was taken to dryness under vacuum. The residue was applied to a silica gel column. Triphenylphosphine oxide was washed from the column with $CHCl_3$, and compound 3 (IAZA) was subsequently eluted with $CHCl_3$:MeOH = 95:5 (v/v). IAZA was recovered as a white crystalline solid (109 mg; 75% yield) by evaporation of the solvent. MP. 122°C. ^{1}H NMR (CH_3OD) δ 7.52 (1H, d, J = 0.9 Hz, C_5-H); 7.12 (1H, d, J = 0.9 Hz, C_4-H); 6.52 (1H, s, C_1'-H); 4.63 (1H, dt, $J_{4',3'}$ = 1.7 Hz, $J_{4',5'}$ = 7.3 Hz, C_4'-H); 4.29 (1H, d, $J_{2',3'}$ = 1.6 Hz, C_2'-H); 4.26 (1H, dd, $J_{3',2'}$ = 1.6 Hz, $J_{3',4'}$ = 1.7 Hz, C_3'-H); 3.44 and 3.51 (2H, dd, $J_{5',5'}$ = 10.2 Hz, $J_{5',4'}$ = 7.3 Hz, C_5'-H). ^{13}C NMR (CH_3OD) δ 144.8 (C_2); 127.7 (C_4); 124.3 (C_5); 96.4 (C_1'); 90.2 (C_4'); 83.1 (C_2'); 78.9 (C_3'); and 5.3 (C_5'). MS (DIP E_V = 70 eV) 355 (M^+; 3%). Exact mass 354.9624, calculated 354.9624 for $C_8H_{10}N_3O_5I$.

1-(5-[^{125}I]Iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole, [^{125}I]-3 (^{125}I-IAZA)

Na[^{125}I]I (29 MBq) was transferred into a Reacti-vial® and evaporated to dryness. Dry dimethyl formamide (200 μl) contain-

ing IAZA 3 (1.6 mg) was added to the dry residue, and the capped vial was heated at 70°C for 3.5 hr. The vial was cooled, after which the reaction mixture was applied to a semipreparative HPLC column for isolation of ^{125}I-IAZA. The radiochemical yield in the exchange reaction was 75%–80% with ^{125}I-labeled iodide as the only other major radioactive contaminant species detected. The radioactive fraction corresponding in retention time to authentic IAZA was collected and dried under vacuum. The product (6.57 GBq/mmol) was stored as a dry film in multidose vials and reconstituted with sterile saline prior to use. The analysis of reconstituted samples by radio-HPLC indicated greater than 99% chemical and radiochemical purity and little or no decomposition over 2 wk when stored at 5°C.

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