Imaging Tumor Hypoxia and Tumor Perfusion

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Tumor perfusion and oxygenation status have been suggested as factors which may influence treatment outcome in cancer patients. Nuclear medicine assays of tumor perfusion [^{99m}Tc-hexamethylpropylenamine oxime (HMPAO)] and tumor hypoxia [^{123}I-iodoazomycin arabinoside (IAZA)] have recently been developed and described. We report on measurements of perfusion and oxygenation status of 27 tumors in 22 patients using these probes. An inverse correlation between tumor uptake of HMPAO and IAZA was measured ($p < 0.05$), with severe perfusion deficit usually associated with an increased uptake of the hypoxic marker. This trend was observed for limited stage small-cell lung carcinoma, squamous-cell carcinoma of the head and neck, soft-tissue sarcoma, brain metastases from small-cell lung carcinoma and adenocarcinoma of the prostate as a group, but not for glioblastoma multiforme. Whereas each imaging agent can yield information about the physiological status of tumor and normal tissue, the information resulting from their combined use could be important in cancer therapy.

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It has been recognized for over 30 yr that the presence of hypoxic cells within some tumors may play a role in their resistance to radiotherapy and chemotherapy (*1,2*). Several groups have attempted to develop useful techniques for measuring tissue hypoxia, although no simple method has yet been established. Measurements by invasive oxygen electrodes (*3,4*), by nuclear magnetic resonance spectroscopy of phosphorus signals associated with cellular energy metabolism (*5,6*) and by uptake of radiolabeled bioreductive drugs (e.g., misonidazole) into hypoxic tissues (*7,8*) have been proposed. This latter technique involves the systemic administration of a radiolabeled bioreductive drug which can be reduced within cells to reactive intermediates that bind to cellular molecules at rates inversely proportional to intracellular oxygen concentration. Carbon-14 and tritium-labeled radiosensitizers have been shown to selectively bind to hypoxic regions of solid tumors in both rodents and humans (*8–10*).

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Recently, an analogue of misonidazole labeled with ^{18}F was shown to selectively bind to hypoxic cells within tumors and myocardium (*11,12*). However, imaging with this agent is limited to those centers with positron emission tomography (PET) systems, which precludes its routine use in most clinical centers. Our group has reported the synthesis of a novel misonidazole analogue, iodoazomycin arabinoside (IAZA), which can be labeled with ^{123}I (*13,14*). The binding of this compound to the acid insoluble fraction of EMT-6 cells has been shown to be strongly dependent, at 37°C, on the degree of hypoxia (*13*). Iodine-123 can be detected by both planar scintigraphy and single-photon emission computer tomography (SPECT) with nuclear medicine equipment available in most imaging departments. Furthermore, the 13-hr half-life of ^{123}I compared to the 100-min of ^{18}F allows for delayed imaging to be performed. Animal and preliminary human data have indicated that optimum tumor-to-background ratios are obtained after two to three pharmacologic half-lives during which time clearance of unbound compound from oxic tissues occurs (*13,14*).

Regional tissue perfusion can also be an indicator of tissue viability, and this property is commonly used in the evaluation of ischemic heart disease (*15*). Several groups have shown ^{99m}Tc-hexamethylpropylenamine oxime (HMPAO) to be an effective indicator of tissue and tumor perfusion in animals (*16*) and in humans (*17*). Studies with the Dunning rat prostate carcinoma (R3327-AT) have demonstrated an inverse relationship between HMPAO and IAZA uptake in untreated tumors and in those treated with photodynamic therapy (PDT) (*18*). To assist in understanding the relationship between tumor perfusion and tumor hypoxia in man, we made a qualitative comparison of HMPAO and IAZA distribution in patients with a variety of tumors at different sites in which significant hypoxic fractions were expected.

METHODS

Patient Population

Twenty-two patients (16 male and 6 female) with primary small-cell lung cancer (SCLC, n = 4), glioblastoma multiforme (n = 4), head and neck squamous-cell carcinoma (n = 6), brain metastases from SCLC (n = 2), soft-tissue sarcoma (n = 5) and prostatic carcinoma (n = 1) were entered into the study after giving informed written consent. The protocol had previously been approved by the institutional ethics committee. The anatom-

TABLE 1
Distribution of IAZA and HMPAO Uptake According to Tumor Pathology and Number of Sites

	No. of tumor sites	IAZA uptake			HMPAO uptake		
		Decreased	Equal	Increased	Decreased	Equal	Increased
SCLC	6	1	1	4	2	3	1
Glioblastoma multiforme	4	0	4	0	4	0	0
Brain metastasis	2	0	1	1	1	1	0
Prostate	1	0	1	0	0	1	0
Sarcoma	8	0	2	6	5*	0	3
Head and Neck	6	0	4	2	1*	2	3
Total	27	1	13	13	13	7	7

*Indicates one patient with doughnut pattern.

ical location of the tumors was defined by CT imaging, radiography and/or clinical examination.

Imaging Protocol

Imaging was performed with a General Electric 400 AC gamma camera system (General Electric, Milwaukee, WI) interfaced to a Picker PCS 512 computer system (Picker International, Bedford, OH).

IAZA Study

Unlabeled IAZA (1-(5'-iodo-5'deoxy-β-D-arabinofuranosyl)-2-nitroimidazole) was prepared as described elsewhere (*14*). Iodine-123, 1,110 MBq (30 mCi), as NaI (Nordion International Ltd., Vancouver, Canada) in 0.1 ml solution of 0.1 *M* NaOH contained in a 3-ml V-vial was evaporated to dryness at 40°C with a stream of nitrogen gas. The dry residue was treated with 1.3 mg of IAZA and 3.1 mg of pivalic acid as a solution in 100 μl of aqueous methanol and analyzed by high-pressure liquid chromatography (HPLC). The chemical purity of the crude product was 96% with a 4% impurity identified as 1-(β-D-arabinofuranosyl)-2-nitroimidazole, the hydrolysis product of IAZA. The radiochemical purity was measured as 92.6% with a 4% impurity identified as ^{123}I-iodide. The sample was purified by HPLC and the solvent removed in vacuo. The patient dose was prepared by dissolving the ^{123}I-IAZA in 5.7 ml of sterile saline containing 10 mg of unlabeled IAZA and filtering the solution into a sterile multidose vial. The purified sample had no detectable chemical impurities and a >99% radiochemical purity when analyzed by HPLC.

Because this was a novel compound and because this class of drug has previously shown neurotoxicity, ^{123}I-IAZA [3.9–9.3 mCi (145–343 MBq)] was given by slow intravenous infusion over 5–10 min. In the later phases of the study, after no toxicity had been observed, the radiopharmaceutical was given by bolus injection. Lugol's iodine was administered orally for 3 days prior to imaging to block thyroid uptake of free radioiodine. As previously described (*14*), anterior and posterior planar static images were obtained of the area of interest at 1 and 16–24 hr postinjection. SPECT imaging of the area of interest was performed between 16–24 hr postinfusion. We have reported the results of IAZA imaging, but not data from the PAO study, from four patients in this series elsewhere (*14*).

HMPAO Study

HMPAO images of tumor perfusion were acquired within 1 wk of acquiring the IAZA image. Anterior and posterior planar images and SPECT images of the area of interest were obtained 30–90 min after intravenous injection of 20 mCi (740 MBq) of HMPAO.

Two independent observers compared the uptake pattern of each of the two radiopharmaceuticals. The degree of uptake was graded by the following criteria:

1: Increased (tumor uptake higher than background).
2: Nonavid (tumor uptake equal to that of background).
3: Decreased (tumor uptake less than background). Background was defined as uptake in contralateral normal tissue.

The comparative data were tabulated and compared using the chi-square test.

RESULTS

Twenty-seven tumors were identified as known lesions in the 22 patients. Each tumor was analyzed independently and tumor uptake of both imaging agents was scored and compared (Table 1). For the IAZA images, 13 (48%) of the tumors showed increased uptake, 13 tumors (48%) showed equal uptake and 1 tumor (4%) showed decreased uptake. For HMPAO images, 13 tumors (48%) showed decreased uptake, 7 tumors (26%) showed equal uptake and 7 tumors (26%) showed increased uptake. Tumors displaying decreased HMPAO uptake tended to be IAZA-avid (Figs. 1 and 2). In two of the tumors displaying decreased HMPAO uptake, the SPECT images revealed a "doughnut" pattern with enhanced perfusion at the tumor periphery. In tumors displaying this "doughnut" pattern of HMPAO uptake, SPECT IAZA images demonstrated a central avidity which corresponded to the photopenic center of the perfusion image.

Eight of 13 tumors (62%) with decreased HMPAO uptake showed increased IAZA uptake, whereas only 5 of 14 tumors (36%) with normal or increased HMPAO uptake showed increased IAZA uptake (Fig. 3). Considering tumors from all sites, these proportions are not significantly different ($\chi^2 = 1.73$, ns). We observed, however, that none of the four glioblastomas displayed IAZA avidity, despite all having striking perfusion defects. Since these tumors may represent a unique category, we examined the patterns of HMPAO and IAZA uptake excluding glioblastoma multiforme. Eight of nine tumors (89%) with decreased

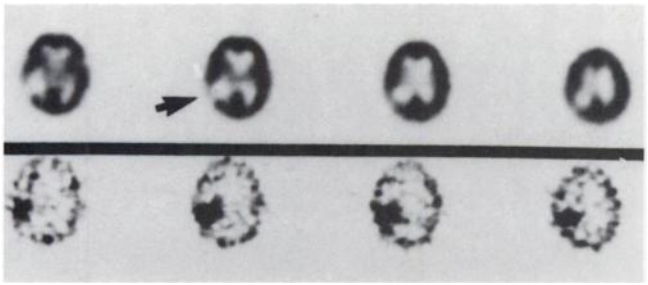

FIGURE 1. Axial SPECT images of a patient with SCLC brain metastasis. The HMPAO study (top row) shows decreased perfusion at the tumor site (arrow) with increased IAZA uptake (bottom row).

HMPAO uptake showed increased IAZA uptake, whereas only 5 out of 14 tumors (36%) with normal or increased HMPAO uptake showed increased IAZA uptake (χ^2 = 6.03, $p < 0.05$).

DISCUSSION

The patterns of IAZA uptake reported in this study are concordant with human tumor hypoxic fraction data measured with ^{3}H-misonidazole (*10*). In that study, 12 of 27 tumors (44%) showed evidence of significant hypoxic fractions by levels of bound ^{3}H-misonidazole significantly higher than those of background. The distribution of tumor sites investigated in the ^{3}H-misonidazole and this study were different. Nevertheless, in both studies a large proportion of the SCLCs investigated showed avidity for these hypoxic markers. The inverse correlation between human tumor perfusion and hypoxic marker uptake is consistent with our study with animal tumors (*18*). In that study, rat prostate carcinomas (R3327-AT) were investigated before and after photodynamic therapy, a procedure known to induce perfusion shutdown in treated tissues (*6,19*). These studies demonstrated that the extent of perfusion deficit and tumor cell hypoxia in PDT-treated tumors was larger than that found in nontreated tumors.

In Figure 1, there is IAZA avidity at the site of a cerebral metastasis in a patient with SCLC, associated with a discordant HMPAO image. It is unlikely that the IAZA uptake reflects breakdown of the blood-brain barrier. The negligible IAZA uptake found in gliomas, and the absence

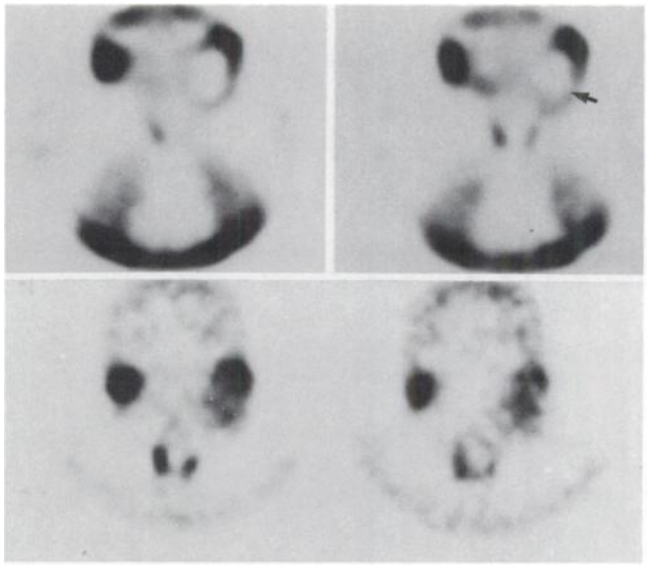

FIGURE 2. Coronal SPECT images of a patient with a bulky neck node metastasis from a squamous-cell carcinoma of unknown primary site. The HMPAO study (top row) shows decreased perfusion in the lymph node metastasis (arrow) with corresponding IAZA avidity (bottom row). Iodine-123 uptake in the salivary glands is presumably due to partial deiodination (*14*).

of HMPAO uptake, make it unlikely that such a nonspecific uptake mechanism, normally demonstrated by glucoheptonate scintigraphy, is responsible for the patterns of uptake reported in this paper.

Whereas an inverse correlation between tumor perfusion and hypoxic marker avidity was found in this study, an absolute correspondence between perfusion shutdown and tumor hypoxic fraction was not expected. The extent of perfusion reduction in a specific tumor and/or tissue required to produce significant zones of cells at oxygen levels low enough to promote the reduction and binding of these markers is probably tissue-specific. Endogenous levels of nitroreductases, tissue rates of oxygen utilization, the "quality" of tissue vessels and other biological parameters will impact on such correlations. In four patients with glioblastoma multiforme, we noted that the tumors showed significantly reduced perfusion relative to surrounding normal brain tissue, yet displayed no significant avidity for IAZA. If this result is confirmed by additional studies, this

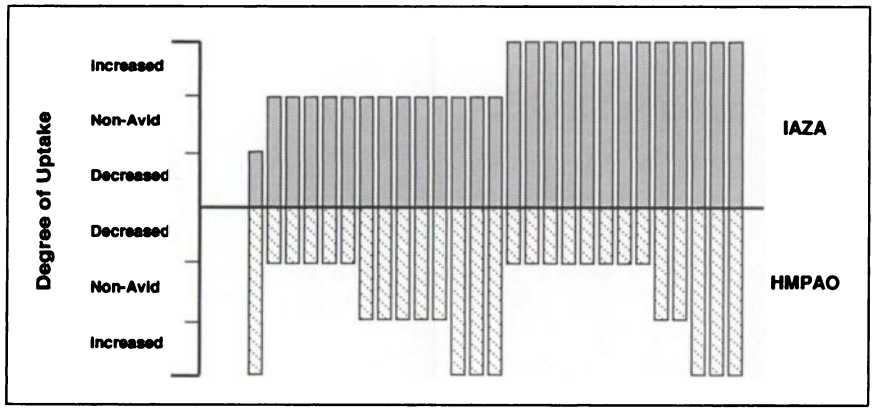

FIGURE 3. Histogram shows the comparative degree of IAZA and HMPAO uptake observed in 27 tumor sites.

could suggest that malignant glioma cells are extremely sensitive to ischemic cell death with viable hypoxic cells being a rarity in these tumors. Additional information is required before realistic modeling of tumor/tissue blood flow and viable hypoxic fraction as measured by IAZA avidity can be performed.

The 16-hr imaging time for IAZA was utilized because of a late afternoon injection. Studies are planned to evaluate the 8-hr to 16-hr postinjection imaging period.

Reduced regional HMPAO uptake is unable to distinguish between necrotic or hypoxic tissue and is likely to underestimate the extent of viable tumor. The patterns of IAZA uptake shown in Figure 1 suggest that this marker can discriminate viable hypoxic tumor, a parameter which could have significant prognostic potential. The inverse correlation of uptake of the two radiopharmaceuticals suggests a complex relationship between impaired tissue perfusion and the presence of viable hypoxic cells. The data also suggest that both HMPAO and IAZA measurements of tumor physiology may provide additional information to those prescribing cancer treatments.

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REFERENCES

1. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OCA. Concentration of oxygen dissolved in tissues at time of irradiation as a factor in radiotherapy. *Br J Radiol* 1953;26:638–648.
2. Moulder JE, Rockwell S. Tumor hypoxia: its impact on cancer therapy. *Cancer Metastasis Rev* 1987;5:313–341.
3. Cater DB, Silver IA. Quantitative measurements of oxygenation in normal tissues and in the tumors of patients before and after radiotherapy. *Acta Radiol* 1960;53:233–256.
4. Gatenby RA, Kessler HB, Rosenblum JS, et al. Oxygen distribution in squamous cell carcinoma metastases and its relationship in outcome of radiation therapy. *Int J Radiat Oncol Biol Phys* 1988;14:831–838.
5. Rofstad EK, DeMuth P, Fenton BM, Ceckler T, Sutherland RM. P-31 NMR spectroscopy and HbO_2 cryospectrophotometry in prediction of tumor radioresistance caused by hypoxia. *Int J Radiat Oncol Biol Phys* 1989;16:919–923.
6. Chapman JD, McPhee MS, Walz N, et al. Nuclear magnetic resonance spectroscopy and sensitizer-adduct measurements of photodynamic therapy-induced ischemia in solid tumors. *J Nat Can Inst* 1991;83:1650–1659.
7. Chapman JD. Hypoxic sensitizers: implications for radiation therapy. *N Engl J Med* 1979;301:1429–1432.
8. Chapman JD, Franko AJ, Sharplin J. A marker for hypoxic cells in tumors with potential clinical applicability. *Br J Cancer* 1981;43:546–550.
9. Urtasun RC, Chapman JD, Raleigh JA, Franko AJ, Koch CJ. Binding of ^{3}H-misonidazole to solid human tumors as a measure of tumor hypoxia. *Int J Radiat Oncol Biol Phys* 1986;12:1263–1267.
10. Chapman JD, Urtasun RC, Franko AJ, Raleigh JA, Meeker BE, McKinnon SA. The measurement of oxygenation status of individual tumors. In: "Prediction of Response in Radiation Therapy: The Physical and Biological Basis". *Am Assoc Phys Med Symposium Proceedings, No. 7 (Part 1)*; 1989:49–60.
11. Koh W-J, Rasey JS, Evans ML, et al. Imaging of hypoxia in human tumors with [F-18]fluoromisonidazole. *Int J Radiat Oncol Biol Phys* 1992;22:199–212.
12. Shelton ME, Dence CS, Hwang D-R, Welch MJ, Bergmann SR. Myocardial kinetics of fluorine-18-misonidazole: a marker of hypoxic myocardium. *J Nucl Med* 1989;30:351–358.
13. Mannan RH, Somayaji VV, Lee J, Mercer JR, Chapman JD, Wiebe LI. Radioiodinated 1-(5-iodo-5-decoxy-β-D-arabinofuranosyl)-2-nitroimidazole (iodoazomycin arabinoside: IAZA): a novel marker of tissue hypoxia. *J Nucl Med* 1991;32:1764–1770.
14. Parliament MB, Chapman JD, Urtasun RC, et al. Non-invasive assessment of human tumour hypoxia with ^{123}I-iodoazomycin arabinoside: preliminary report of a clinical study. *Br J Cancer* 1992;65:90–95.
15. Kloner RA, Przyklenk K. Hibernational and stunning of the myocardium [Editorial]. *N Engl J Med* 1991;325:1877–1879.
16. Suga K, Honma Y, Uchisako H, et al. Assessment of ^{99m}Tc-HMPAO tumour scintigraphy using VX-2 tumours implanted in a lower limb muscle of rabbits. *Nucl Med Commun* 1991;12:611–619.
17. Powell NP, McCready VR, Tait D, et al. Technetium-99m HMPAO and SPECT in the assessment of blood flow in human lung tumours. *Br J Cancer* 1989;59:135–141.
18. Moore RB, Chapman JD, Mercer JR, et al. Measurement of photodynamic therapy induced hypoxia in the Dunning prostatic tumor model by ^{123}I-Iodoazomycin arabinoside. *J Nucl Med* 1992;34:405–413.
19. Moore RB, Chapman JD, Mokrzanowski AD, Arnfield MR, McPhee MS, McEwan AJB. Non-invasive monitoring of photodynamic therapy with ^{99m}Tc-HMPAO. *Br J Cancer* 1992;65:491–497.