telman A. Photoradiation therapy for the treatment of malignant tumors. *Cancer Res* 1978;38:2828–2835.
7. Moan J, Reng Q, Eversen JF, Berg K, Western A, Rimington C. Photosensitizing efficiencies, tumor and cellular uptake of different drugs relevant for photodynamic therapy of cancer. *Photochem Photobiol* 1987;46: 713–721.
8. Henderson BW. Photodynamic therapy: coming of age. *Photodermatology* 1989;6:200–211.
9. Hilf R, Murant RS, Narayanan U, Gibson SL. Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyrin derivative-induced photosensitization in R3230AC mammary tumors. *Cancer Res* 1986;46:211–217.
10. Davson H, Ponder E. Photodynamically induced cation permeability and its relation to hemolysis. *J Cell Comp Physiol* 1990;15:67–74.
11. Morliere P, Santus R, Maziere JC, et al. Lysosomes as primary targets of Photofrin II photosensitization in cultured human fibroblasts: a kinetic, spectral and topographic investigation by microspectro fluorometry on single living cells. *J Cell Pharmacol* 1991;2:143–151.
12. Chaudhuri I, Keck RW, Selman SH. Morphological changes of tumor microvasculature following hematoporphyrin derivative sensitized photodynamic therapy. *Photochem Photobiol* 1987;46:823–827.
13. Ben-Hur E, Heldman E, Crane SW, Rosenthal I. Release of clotting factors from photosensitized endothelial cells: a possible trigger of blood vessel occlusion by photodynamic therapy. *FEB Lett* 1988;236:105–108.
14. Fingar VH, Wieman TJM, Doak KW. Role of thromboxane and prostacyclin release on photodynamic therapy-induced tumor destruction. *Cancer Res* 1990;50:2599–2603.
15. Castellani A, Pace GP, Concioli M. Photodynamic effect of hematoporphyrin on blood microcirculation. *J Path Bact* 1963;86:99–102.
16. Selman DH, Kreimer-Birnbaum M, Klaunig JE, Goldblatt PJ, Keck RW, Britton SL. Blood flow in transplantable bladder tumors treated with hematoporphyrin derivative and light. *Cancer Res* 1984;44:1924–1927.
17. Henderson BW, Waldow SM, Mang JS, Potter WR, Malone PB, Dougherty TJ. Tumor destruction and kinetics of tumor cell death in two experimental mouse tumors following photodynamic therapy. *Cancer Res* 1985; 45:572–576.
18. Starr WM, Marijnissen HPA, van den Berg-Blok AE, Versteeg JAC, Frankeen KAP, Reinhold HS. Destruction of rat mammary tumor and normal tissue microcirculation by hematoporphyrin derivative photoradiation observed *in vivo* in sandwich observation chambers. *Cancer Res* 1986;46:2532–2540.
19. Gonzalez W, Arnfield MR, Meeker BE, et al. Treatment of Dunning R3327-AT rat prostate tumors with photodynamic therapy in combination with misonidazole. *Cancer Res* 1986;46:2858–2862.
20. Hirsh BD, Walz NC, Meeker BE, et al. Photodynamic therapy-induced hypoxia in rat tumors and normal tissues. *Photochem Photobiol* 1987;46: 847–852.
21. 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 Natl Can Inst* 1991;83:1650–1659.
22. Moore RB, Chapman JD, Mokrzanowski AD, Arnfield MR, McPhee MS, McEwan AJ. Non-invasive monitoring of photodynamic therapy with ^{99m}Tc-HMPAO scintigraphy. *Br J Cancer* 1992;65:491–497.
23. Mannan RH, Somayaji VV, Lee J, Mercer JR, Chapman JD, Wiebe LI. Radioiodinated 1-(5-iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole (iodoazomycin arabinoside: IAZA): a novel marker of tissue hypoxia. *J Nucl Med* 1991;32:1764–1770.
24. Chapman JD, Lee J, Meeker BE. Adduct formation by 2-nitroimidazole drugs in mammalian cells: optimization of markers for tissue oxygenation. In: Adams GE, et al., eds. *Selective activation of drugs by redox processes*. New York: Plenum Press; 1990:313–323.
25. Parliament MB, Chapman JD, Urtasun RC, et al. Non-invasive assessment of human tumor hypoxia with ^{123}I-iodoazomycin arabinoside: preliminary report of a clinical trial. *Br J Cancer* 1992;65:90–95.
26. Thorndyke C, Meeker BE, Thomas C, Lakey WH, McPhee MS, Chapman JD. The radiation sensitivities of R3327-H and R3327-AT prostate adenocarcinomas. *J Urology* 1985;134:191–198.
27. Ballinger JR, Reid RH, Gulenchyn KY. Radiochemical purity of ^{99m}Tc-HMPAO [Abstract]. *J Nucl Med* 1988;29:572.
28. Chapman JD. The cellular basis of radiotherapeutic response. *Rad Phys Chem* 1984;24:283–291.

EDITORIAL

Second Generation Hypoxia Imaging Agents

The contribution by Moore et al. (*1*) comes from the same laboratory that first proposed hypoxia imaging in 1979 (*2*) and describes imaging of both nontreated and photodynamic therapy (PDT) treated prostate tumors in rats with iodoazomycin arabinoside (^{123}I-IAZA). Tumor perfusion in these same tumors was measured with ^{99m}Tc-HMPAO. Part of the clinical interest in PDT involves the development of techniques for the treatment of human prostatic adenocarcinomas (*3*). This therapy results in membrane damage dependent upon the concentration of the photosensitizing drug, the activating light source and the intracellular concentration of oxygen (*4*). If there is insufficient oxygen present, singlet oxygen ($^1O_2$) cannot be produced, and the desired disruption of mitochondrial oxidative phosphorylation, membrane leakiness, lysosomal autolysis and endothelial cell damage are not achieved. One limitation of PDT is that it results in formation of a hypoxic cell fraction within the tumor being treated, which is then resistant to further PDT treatment. Previous work from these authors demonstrated that PDT-induced tumor ischemia was dependent upon the light dose used and the time of observation after the treatment (*3*). The microvasculature was the most sensitive target for PDT and the consequential events after PDT include blood stasis, tumor tissue ischemia and secondary tumor cell hypoxic death. Therefore, measurement of oxygenation status and perfusion within the tumor should be useful in monitoring the potential success of PDT. An iodinated azomycin nucleoside hypoxic cell marker that displayed greater sensitivity to O_2 levels, thus allowing for closer monitoring of the progress of PDT in tumors, would be a valuable contribution.

The report concludes that ^{123}I-IAZA and other iodinated azomycin nucleosides show promise for monitoring tumor oxygenation status, in

Received Dec. 10, 1992; accepted Dec. 10, 1992.
For correspondence or reprints contact: Joseph E. Biskupiak, Imaging Research Laboratory, RC-05, University of Washington, Seattle, WA 98195.

particular the effectiveness of interstitial PDT treatments where perfusion-shutdown is a major mechanism of tumor response. The results demonstrate that PDT-induced ischemia could be monitored with ^{123}I-IAZA and loss of tumor perfusion could be verified with ^{99m}Tc-HMPAO. Unfortunately, a light dose-dependent change in marker uptake was not observed in the study with either ^{123}I-IAZA or ^{99m}Tc-HMPAO. As pointed out by the authors, previous studies with ^{3}H-MISO as the hypoxic cell marker showed a 2.4 greater increase (compare to 1.53 for ^{123}I-IAZA) of retained drug in PDT-treated tumors compared to untreated controls (*3*). Further, the tumor-to-brain ratios of bound ^{3}H-misonidazole (^{3}H-MISO) in both control and PDT-treated tumors were larger than those measured in the study with ^{123}I-IAZA. More importantly, these ratios displayed a light dose dependency.

The iodinated azomycin nucleosides are a new and interesting class of hypoxic cell markers, that appear to have an affinity for the membrane transporter of nucleosides in vivo. Their octanol-water partition coefficients are reported in the range of 2 to 6, which allows them to cross biological membranes more easily than MISO and fluoromisonidazole (FMISO) and consequently increase their concentration in hypoxic tissue (*5,6*). The advantages as well as complications of this difference in membrane transport are discussed below. The other feature of hypoxic cell markers, their single electron reduction potential, is responsible for the O_2-dependent formation of reactive intermediates forming covalent bonds within hypoxic tissue. This is the process that results in their selective trapping and will require further development within the iodinated azomycin nucleoside class of hypoxic cell markers. This is suggested by the inability of ^{123}I-IAZA to detect a light dose-dependent change in uptake in the PDT study.

The partition coefficient and reduction potential of 2-nitroimidazoles are responsible, respectively, for their ability to cross biological membranes and undergo bioreduction, resulting in trapping within hypoxic tissue. Any efforts to improve on these compounds as hypoxia imaging agents has to address changes in these two physical properties. Increasing the lipophilicity could have the undesired effect of increasing nonspecific binding and decreasing body clearance. Alteration of the nitro reduction potential may result in a molecule possessing enhanced sensitivity to the residual O_2 concentration in tissues. There is no simple prediction of which direction the reduction potential should be altered. Radiopharmaceutical developments should look at changes in both directions.

The most helpful way of modifying how a nitroimidazole crosses membranes is also difficult to predict. The nucleoside derivative may be contrasted to FMISO. FMISO exhibits a single mode of uptake by diffusion into cells followed by selective retention in hypoxic tissue. IAZA exhibits dual mode uptake. In addition to diffusion followed by reduction in hypoxic tissue, its uptake also involves selective membrane transport of nucleosides. FMISO uptake in normoxic tissue, other than kidney and liver, rapidly equilibrates with plasma drug. Selective retention in hypoxic tissue cannot be imaged until 2 hr or later postinjection. The equilibration of FMISO with plasma has been measured in our laboratory in nonmalignant tissues; the mean FMISO ratio from over 1000 samples was 1.035, and 90% of the ratios fell below 1.31 (*7*). From these data, we conservatively estimate that hypoxia is inferred by a tumor-to-plasma ratio value of $\geq$1.4 at 2 hr or more postinjection. The uptake of IAZA and other iodinated azomycin nucleosides are not likely to fit these empirical observations due to the second mechanism of uptake, selective membrane transport of nucleosides (*5*), which varies from tissue to tissue, independent of its oxygen status. This dual mode of uptake may make quantitative interpretation of azomycin nucleosides as hypoxic cell markers more complicated.

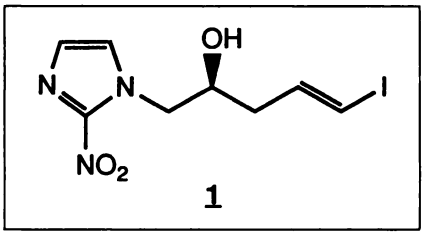

FIGURE 1. Schematic representation of IVM.

We have also been interested in the development of an iodinated derivative of MISO as well as other hypoxic cell markers that possess greater sensitivity toward varying levels of O_2 concentrations in tissues. Our efforts to develop an iodinated derivative of MISO have resulted in the synthesis and evaluation of iodovinylmisonidazole (IVM, (*1*)) (Fig. 1), which has been labeled with both ^{131}I and ^{123}I and tested in vitro and in vivo (mice and dogs) as a hypoxia imaging agent. IVM was compared to FMISO and exhibited similar oxygen dependency of binding in both tumor cells and rat myocytes. Electron affinity, i.e., the reduction potential of the nitro group of the nitroimidazole, should not be significantly different between IVM and FMISO. This was proven by the data showing that the O_2 level which inhibited binding of the two drugs was similar. In spite of IVM's increased lipophilicity (octanol-water partition coefficient of 2.52 versus 0.40 for FMISO), nonspecific protein building was negligible and deiodination in vivo was not a problem (*8*).

Our recent research has focused on development of hypoxic cell markers exhibiting more rapid oxygen-sensitive bioreduction, than the two or more hours optimal for FMISO. Our goal is a hypoxia imaging agent that can be used in patients with acute ischemia and mild ischemia in the myocardium. Such an agent could also be useful in exhibiting a light dose-dependent change in marker uptake in PDT. An oxygen concentration of 1000 ppm is suffi-

cient to inhibit binding of FMISO and IVM by 50% relative to anoxic tissue. In order to achieve binding at higher O_2 concentrations, the oxygen-dependency of binding must be altered by modifying the nitro reduction potential. Binding takes place only in hypoxic tissue because in normoxic tissue, O_2 is a better electron acceptor and promotes back reaction to parent drug. In the intracellular space, the initial fast reaction between R-NO_2 and electrons from electron transport to give the one electron reduction product, R-NO_2^-, is probably not oxygen-dependent and will not be altered by changes in the reduction potential of the nitro group. The reoxidation step of the futile cycle, however, is probably oxygen-dependent and will change with changes in the reduction potential of the nitro group. Once a molecule is reduced to the two electron product, it is locked into the remainder of a metabolic path which leads to trapping within hypoxic tissue.

Alteration of the reduction potential of the nitro group of nitroimidazoles can be achieved via introduction of electron-withdrawing or electron-donating groups to the nitroimidazole ring. Introduction of a hydroxymethyl (electron-donating) or a carboxylic acid-ester (electron-withdrawing) group on the ring would also affect lipophilicity. Our experience with FMISO and IVM does not indicate any beneficial effect to increasing the partition coefficient. Increasing the partition coefficient may even cause whole-body clearance of the drug to decrease, which might require imaging at even later times postinjection.

Future improvements in hypoxic cell markers based on 2-nitroimidazoles need to focus on alteration of the reduction potential of the nitro group to achieve sensitivity to higher oxygen levels. This modification, coupled with changes in lipophilicity, may result in development of new hypoxia imaging agents that exhibit more rapid oxygen-sensitive bioreduction and more favorable whole-body clearance.

Joseph E. Biskupiak
Kenneth A. Krohn
University of Washington
Seattle, Washington

REFERENCES

1. Moore RB, Chapman JD, Mercer JR, et al. Measurement of PDT-induced hypoxia in Dunning prostate tumors by ^{123}I-iodoazomycin arabinoside. *J Nucl Med* 1993;34:000–000.
2. Chapman JD. Current concepts in cancer. Hypoxic sensitizers—implications for radiation therapy. *New Engl J Med* 1979;301:1429–1432.
3. Gonzalez S, Arnfield MR, Meeker BE, et al. Treatment of Dunning R3327-AT rat prostate tumors with photodynamic therapy in combination with misonidazole. *Cancer Res* 1986; 46:2858–2862.
4. Chapman JD, Stobbe CC, Arnfield MR, Santus R, McPhee MS. The effectiveness of short-term versus long-term exposure to Photofrin II in killing light-activated tumor cells. *Radiat Res* 1991;128:82–89.
5. Mannan RH, Somayaji VV, Lee J, Mercer JR, Chapman JD, Wiebe L. Radioiodinated 1-(5-iodo-5-deoxy-b-D-arabinofuronosyl)-2-nitroimidazole (iodoazomycin arabinoside: IAZA): a novel marker of tissue hypoxia. *J Nucl Med* 1991;32:1764–1770.
6. Mannan RH, Mercer JR, Wiebe L, Kumar P, Somayaji VV, Chapman JD. Radioiodinated azomycin pyranoside (IAZP): a novel non-invasive marker for the assessment of tumor hypoxia. *J Nucl Biol Med* 1992;36:60–67.
7. Koh WJ, Rasey JS, Evans ML, et al. Imaging of hypoxia in human tumors with [F-18]fluoromisonidazole. *Int J Radiat Oncol Biol Phys* 1991;22:199–212.
8. Biskupiak JE, Grierson JR, Rasey JS, Martin GV, Krohn KA. Synthesis of an (iodovinyl) misonidazole derivative for hypoxia imaging. *J Med Chem* 1991;34:2165–2168.