INVITED COMMENTARY

On Measuring Hypoxia in Individual Tumors with Radiolabeled Agents

Recently, the National Institutes of Health have identified functional imaging as an important focus for research. In addition, the number of markers available for measuring tumor genotype and phenotype is rapidly increasing, so that the biologic basis of several imaging procedures can be investigated in greater depth. The information contained within PET and SPECT images of radiolabeled markers can provide a sensitive, noninvasive assay for some tumor properties important for cancer diagnosis and treatment planning. Such images, derived from the administration of radiolabeled compounds to patients, are much affected by the pharmacokinetics of these compounds in individual hosts. Some general principles have been established. For example, the intravenous administration of a radiodiagnostic agent is followed by rapid distribution to all tissues in relation to their blood volume and flow rate and subsequent penetration into tissue by both diffusion and active transport mechanisms. This early distribution phase is followed by excretion of the tracer itself and its metabolites, some of which retain the radioactive signal. In general, hydrophilic agents are excreted preferentially by the kidneys and bladder, whereas lipophilic agents are excreted by the hepatobiliary system. Mathematic models have been developed to describe the absolute and relative tissue distributions of various radiolabeled agents as a function of time after their administration. Because most imaging procedures use very low concentrations of the chemical marker, it is the distribution and retention of only the radiolabel that are usually measured. If the proportion of marker specific to a cancer phenotype or genotype is small relative to the total activity delivered to the target tissue, quantification of the marker in tomographic images becomes problematic.

Hypoxic microenvironments of solid tumors contain clonogenic cells that are resistant to being killed by radiation and less accessible to the delivery of some cancer drugs. In addition, this stressful environment promotes cell mutation and selects for hardy and metastatic phenotypes. Because hypoxic fraction (HF) has been shown to predict animal and human tumor response to radiation (*1*,*2*), measurement of HF in individual patients could govern the prescription of novel hypoxia-targeted therapy, including increased-dose "painting" to resistant tumor regions by conformal and intensity-modulated radiotherapy (*3*). Several radiolabeled markers of viable hypoxic cells in solid tumors have been developed, and some of these markers have been tested clinically (*4*). The first clinical imaging studies investigating hypoxia in solid tumors used ^{123}I-labeled iodoazomycin arabinoside (IAZA) with SPECT (*5*) and ^{18}F-labeled fluoromisonidazole (FMISO) with PET (*6*). Although these 2 agents exhibited similar hypoxia-marking kinetics in vitro and their uptake was efficiently visualized in tumor sections by autoradiography (when the unreacted marker was washed away), the SPECT and PET images acquired from human cancer appeared to give quite different results. The percentage of human tumors with a significant HF as defined by SPECT and PET was approximately 40% and approximately 97%, respectively, with the difference likely being an artifact of the imaging procedures (*4*). If all human cancer types that require radiotherapy have significant HFs, then this tumor phenotype should be measured with the highest possible spatial resolution for precision application of an additional dose to radioresistant subvolumes. If only 30%–50% of human cancer has a significant HF, this tumor phenotype should also be determined individually and used to guide the prescription of targeted therapy to only selected patients, because some additional toxicity might be expected.

The quantification of hypoxia-specific uptake of marker at early times after administration will be confounded by the abundance of marker delivered to, but not reacted with or excreted from, the tumor and surrounding tissues. The expected pharmacokinetics of this hypoxia-imaging procedure have been discussed (*4*,*7*). A study was performed with ^{99m}Tc-exametazime (Ceretec; Nycomed Amersham Imaging, Princeton, NJ) and IAZA to determine if tumor perfusion would complicate the interpretation of hypoxia-specific signal (*8*). The study showed that human tumors could be hypoperfused, normally perfused, or hyperperfused relative to the surrounding normal tissues and that hypoxia was more frequently associated with decreased tumor perfusion. However, this relationship between tumor hypoxia and hypoperfusion was not absolute. This and other factors could complicate the noninvasive measurement of tumor hypoxia by radiodiagnostic agents, especially those labeled with short-lived isotopes that require measurement at early times.

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measurements of metabolically active volume, blood flow, blood volume, and hypoxia made with [^{18}F]FDG, [^{15}O]H_2O, [^{15}O]CO, and [^{18}F]fluoroerythronitroimidazole (FETNIM), respectively, in 8 patients with untreated head and neck cancer. The FDG images were acquired before the other assays and used to guide region-of-interest analyses of the other agents. The blood flow to these tumors was 5–30 times greater than that to muscle, although blood volumes were similar. FETNIM levels in muscle were always lower than in blood. FETNIM levels in tumors were always higher than in muscle and, in 4 of 8 patients, were higher than in blood. This variable uptake of the hypoxia marker into the different tumors appeared to be governed by blood flow, at least in the early phase of tissue accumulation. The authors state that their results with FETNIM compare favorably with those reported previously for FMISO, but no advantage was apparent. Unfortunately, no independent measurements of tumor oxygenation or radioresistance were performed for correlation with FETNIM avidity. This is a common deficiency of much of the recent hypoxia marker research performed on both animal and human tumors and relates to the current lack of a gold standard for measuring this tumor property.

Attempts have been made to correlate percentage injected dose per gram (%ID/g) of hypoxia marker retained in animal tumors with microelectrode measurements of partial pressure of oxygen (pO_2), OxyLite (Oxford Optronix Ltd., Oxford, U.K.) pO_2 luminescence, and ^{31}P nuclear magnetic resonance spectroscopy measurements acquired from the same tumors. The azomycin-based hypoxia markers are reduced within viable cells and covalently link to their molecules at rates inversely proportional to intracellular oxygen concentration. The other assays measure tissue oxygenation directly or indirectly but within volumes of up to hundreds of cells. In general, no strong correlation has been found between these parameters of oxygenation and hypoxia marker uptake into the tumors of unperturbed animals (*10*). When the oxygenation status of tumors was modulated widely by vasoactive drugs or different breathing gases, significant correlations between the different oxygen-measuring techniques were observed (*11,12*). However, these pharmacologic modulations alter both delivery of the hypoxic marker and delivery of oxygen to tumor tissue, and appropriate corrections should be made. How, then, should hypoxic marker uptake into tumors be validated experimentally? Laboratory research has concluded that the most appropriate validation technique for animal tumors is some measurement of their radioresistance, such as the in vivo/in vitro assay (*13*). In this manner, a measure of hypoxia marker uptake (%ID/g, tumor-to-blood ratio, tumor-to-muscle ratio, etc.) and radiosensitivity at high doses (surviving fraction after acute doses of 15–20 Gy), at which hypoxia resistance will dominate, can be obtained from individual tumors. Because similar measurements of human tumor radiosensitivity are not feasible, validation of hypoxia marker uptake in clinical studies poses an even greater problem.

Recently, animal studies performed with the micro-PET system at the Memorial Sloan–Kettering Cancer Center (New York, NY) compared hypoxia imaging by [^{124}I]β-D-iodinated azomycin galactopyranoside (IAZGP) and by [^{18}F]FMISO in the same tumor-bearing animals (P.B. Zanzonico et al., unpublished data, 2001). Even in mice, the hypoxia-specific tumor images of FMISO acquired at 1 and 3 h after marker administration were dominated by signal from unbound marker (>90% of the activity in the rest of the body). The same was true for the images of β-D-IAZGP uptake at these early times, but at 24 and 48 h, a far greater proportion (34% and 46%, respectively) of the remaining tracer in the body was found only in tumors. The plasma clearance half-life of FMISO and β-D-IAZGP is about 30–40 min in mice and at least 10 times longer in humans. These data show that quantification of hypoxia-specific signal from PET and SPECT images of hypoxic markers will be optimal at those times when the background is minimal. For this reason, it is unlikely that FETNIM, FMISO, and 2-(2-nitro-1H-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl acetamide (EF5) labeled with ^{18}F, which can only be imaged within a few hours after administration, will produce high-resolution maps that show the radioresistant subregions within tumors to which an extra dose should be delivered. Clinical studies would be wise to investigate those radiodiagnostic agents that can be labeled with isotopes whose physical life-lives permit detection of tumor hypoxia at maximal sensitivity. Of the halogenated hypoxia markers that incorporate azomycin as the bioreducible oxygen sensor, the iodinated markers have this advantage, but they also have drawbacks. Deiodination and subsequent uptake of the radiolabel into thyroid tissue is a disadvantage but can be greatly reduced by adequate thyroid blocking. Although the hypoxia-marking properties of the fluorinated and iodinated markers to tumor cells in vitro and in vivo are almost equal, the precision of imaging the hypoxic microenvironment of tumors will be governed mainly by the non–hypoxia-specific background signal present during image acquisition.

The pharmacokinetics of the metal-labeled hypoxia markers (HL-91, diacetyl-bis(*N*4-methylthiosemicarbazone), azomycin-cyclam derivatives, etc.) are equally important. These agents have much higher specific activities and are consequently administered at only picomolar concentrations. They rapidly distribute to animal tissues at unique levels within 20–30 min and then, with a half-life of 10–12 h, are excreted from all tissues. Consequently, informative images of tumor hypoxia can be acquired with the metal-labeled markers at earlier times, relative to the halogenated markers. But validating that their uptake into individual tumors predicts HF and radioresistance will be equally demanding, especially because the pharmaco-

logic modulation of tumor hypoxia severely modulates the delivery of these markers to the tumors.

It is encouraging that 21 y after the first report on marking hypoxia in tumors by radiolabeled agents (*14*), several markers that can be labeled with different isotopes are now available to optimize this procedure for clinical use in tumor diagnosis and treatment planning. Studies such as that reported by Lehtiö et al. (*9*) will be useful for identifying the optimal agent for measurement of this important tumor phenotype.

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