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. Mathematical 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 HF, 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.

In this issue of The Journal of Nuclear Medicine, Lehtio et al. (9) report

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erythronitroimidazole (FETNIM), re-
spectively, in 8 patients with untreated
head and neck cancer. The FDG im-
ages 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 up-
take of the hypoxia marker into the
different tumors appeared to be gov-
erned 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. Unfor-
nately, 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 hy-
poxia marker research performed on
both animal and human tumors and re-
lates to the current lack of a gold stan-
dard for measuring this tumor property.

Attempts have been made to corre-
late percentage injected dose per gram
(\%ID/g) of hypoxia marker retained in
animal tumors with microelectrode
measurements of partial pressure of
oxygen (pO2). OxyLite (Oxford Optro-
nix Ltd., Oxford, U.K.) pO2 lumines-
cence, and 31P nuclear magnetic reso-
nance spectroscopy measurements
acquired from the same tumors. The
azomycin-based hypoxia markers are
reduced within viable cells and co-
valently link to their molecules at rates
inversely proportional to intracellular
oxygen concentration. The other as-
says measure tissue oxygenation di-
rectly or indirectly but within volumes
of up to hundreds of cells. In general,
no strong correlation has been found
between these parameters of oxygen-
ation and hypoxia marker uptake into
the tumors of unperturbed animals
(10). When the oxygenation status of
tumors was modulated widely by va-
soactive drugs or different breathing
gases, significant correlations between
the different oxygen-measuring tech-
niques were observed (11, 12). How-
ever, these pharmacologic modulations
alter both delivery of the hypoxic
marker and delivery of oxygen to tu-
mor 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 radio-
sensitivity at high doses (surviving
fraction after acute doses of 15–20
Gy), at which hypoxia resistance will
dominate, can be obtained from indi-
vidual tumors. Because similar mea-
surements of human tumor radiosensi-
tivity are not feasible, validation of
hypoxia marker uptake in clinical stud-
ies poses an even greater problem.

Recently, animal studies performed
with the micro-PET system at the Me-
orial Sloan–Kettering Cancer Center
(New York, NY) compared hypoxia
imaging by [124]I]-b-iodinated azomy-
cin galactopyranoside (IAZGP) and by
[18]F]FMISO in the same tumor-bearing
animals (P.B. Zanzonico et al., unpub-
lished 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 im-
ages of β-d-IAZGP uptake at these
early times, but at 24 and 48 h, a far
greater proportion (34% and 46%, re-
spectively) 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 back-
ground 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 aceto-
amide (EF5) labeled with 18F, which
can only be imaged within a few hours
after administration, will produce high-
resolution maps that show the radio-
resistant subregions within tumors
to which an extra dose should be de-
ivered. Clinical studies would be wise
to investigate those radiodiagnostic
agents that can be labeled with iso-
topes 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 io-
dinated markers have this advantage,
but they also have drawbacks. Deiodi-
nation and subsequent uptake of the
radiolabel into thyroid tissue is a dis-
advantage but can be greatly reduced
by adequate thyroid blocking. Al-
though the hypoxia-marking properties
of the fluorinated and iodinated mark-
ers to tumor cells in vitro and in vivo
are almost equal, the precision of im-
ageing the hypoxic microenvironment
of tumors will be governed mainly by
the non–hypoxia-specific background
signal present during image acquisi-
tion.

The pharmacokinetics of the metal-
labeled hypoxia markers (HL-91, di-
acetyl-bis(N4-methylthiosemicarba-
zeone), azomycin-cyclam derivatives,
etc.) are equally important. These
agents have much higher specific ac-
activities and are consequently adminis-
tered at only picomolar concentrations.
They rapidly distribute to animal tis-
sues at unique levels within 20–30 min
and then, with a half-life of 10–12 h,
are excreted from all tissues. Conse-
quently, 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 ra-
dioresistance will be equally demand-
ing, 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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REFERENCES