Imaging Hypoxia in Orthotopic Rat Liver Tumors with Iodine 124–labeled Iodoazomycin Galactopyranoside PET¹

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Purpose:

To evaluate iodine 124 (¹²⁴I)-labeled iodoazomycin galactopyranoside (IAZGP) positron emission tomography (PET) in the detection of hypoxia in an orthotopic rat liver tumor model by comparing regions of high ¹²⁴I-IAZGP uptake with independent measures of hypoxia and to determine the optimal time after injection to depict hypoxia.

Materials and Methods:

The institutional animal care and use committee approved this study. Morris hepatoma tumors were established in the livers of 15 rats. Tumor oxygenation was measured in two rats with a fluorescence fiberoptic oxygen probe. ¹²⁴I-IAZGP was coadministered with the established hypoxia markers pimonidazole and EF5 in nine rats; 12-hour PET data acquisition was performed 24 hours later. Tumor cryosections were analyzed with immunofluorescence and autoradiography. In the four remaining rats, serial 20- and 60-minute PET data acquisition was performed up to 48 hours after tracer administration.

Results:

Oxygen probe measurements showed severe hypoxia (<1 mm Hg) distributed evenly throughout tumor tissue. Analysis of cryosections showed diffuse homogeneous uptake of ¹²⁴I-IAZGP throughout all tumors. The ¹²⁴I-IAZGP distribution correlated positively with pimonidazole ($r = 0.78$) and EF5 ($r = 0.76$) distribution. Tracer uptake in tumors was detectable with PET after 24 hours in seven of nine rats. In rats that underwent serial PET, tumor-to-liver contrast was sufficient to enable detection of hypoxia between 6 and 48 hours after tracer administration. The optimal ratio between signal intensity and tumor-to-liver contrast occurred 6 hours after tracer administration.

Conclusion:

Regions of high ¹²⁴I-IAZGP uptake in orthotopic rat liver tumors are consistent with independent measures of hypoxia; visualization of hypoxia with ¹²⁴I-IAZGP PET is optimal 6 hours after injection.

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Hypoxia has been shown to be a common feature of a wide range of solid tumor types, including breast, prostate, brain, colorectal, pancreatic, gastric, ovarian, lung, renal, head and neck, and melanoma tumors (1–5). In addition, high tumor hypoxia levels have been reported recently in liver metastases from colorectal cancer (6). Tumor hypoxia has been associated with increased cancer aggressiveness and resistance to chemotherapy and radiation treatment. Promising new treatment strategies, such as the use of hypoxia-activated prodrugs and hypoxia-triggered gene therapy, are now being developed (7). Thus, the ability to assess tumor hypoxia in liver tumors would be useful for determining patient prognosis and for selecting patients who would benefit from hypoxia-triggered treatment regimens to improve treatment efficacy.

Techniques used to noninvasively assess tumor hypoxia with magnetic resonance imaging (8) and positron emission tomography (PET) (9–17) are under investigation. Nitroimidazole-based hypoxia-avid PET tracers, such as fluorine 18 (^{18}F) fluoromisonidazole and copper 64 (^{64}Cu) diacetyl-bis-(N^4 -methylthiosemicarbazone), have shown promise in the in vivo assessment of tumor hypoxia (10,12,14,16–18). However, selection of the optimal hypoxia PET tracer will depend on both the sensitivity of the radiotracer to hypoxia and the tumor location with respect to biodistribution of the tracer (19,20). Previous studies have shown high nonspecific intraabdominal tracer levels during the first hours after tracer administration, which suggest that late

imaging might be advantageous (21,22). The nitroimidazole-based compound iodoazomycin galactopyranoside (IAZGP) labeled with the long-lived positron emitter iodine 124 (^{124}I) (4.2-day half-life) has been shown to be a valuable hypoxia tracer in mouse flank tumor xenografts, yielding considerably higher tumor-to-background contrast later (1–2 days) rather than earlier (up to several hours) after administration (12,22). The purpose of our study was to evaluate ^{124}I -IAZGP PET in the detection of hypoxia in an orthotopic rat liver tumor model by comparing regions of high ^{124}I -IAZGP uptake with independent measures of hypoxia and to determine the optimal time after injection to depict hypoxia.

Materials and Methods

Animal Tumor Model

The rat hepatocellular carcinoma cell line Morris hepatoma RH7777 was obtained from the American Type Culture Collection (Rockville, Md) and was maintained in Dulbecco's modified Eagle's medium (Memorial Sloan-Kettering Cancer Center media lab, New York, NY) supplemented with 4.5 g/L glucose, 10% fetal bovine serum, and 5 mmol/L l-glutamine. The cells were maintained at 37°C with 5% carbon dioxide in the air and subcultured twice weekly.

This experimental protocol was approved by the institutional animal care and use committee of our institution. An overview of the experimental setup is shown in Figure 1. Nude rats (weight range, 200–250 g) were housed two per cage and allowed food and water ad libitum. All procedures were performed with the animals anesthetized by means of inhalation of 2% isoflurane and 100% oxygen at a flow rate of 1.5 L/min. Subcutaneous flank tumors were established by injecting approximately 5×10^6 Morris hepatoma cells (RH7777) in phosphate-buffered saline solution (50 μL). When flank tumors had grown to 1 cm in diameter, the animal was sacrificed. Thereafter, the tumors were harvested, cut into small fragments of approximately 2 mm³, and kept in a phosphate-buffered saline

solution on ice. Fifteen other nude rats were then anesthetized and opened ventrally via a midline incision to expose the left, median, and right lobes of the liver. With a pointed 11-blade scalpel lying flat on the liver surface, a 1-cm-long tunnel was punctured into each lobe horizontally just beneath the liver capsule, and approximately 2-mm³ tumor fragments were inserted into each of the resulting superficial pouches. The animals were closed and returned to their cages. Tumor growth was monitored with a dedicated small-animal ultrasonography system (Vevo 770; VisualSonics, Toronto, Ontario, Canada). In animals in which the implanted tumor fragments disseminated into the peritoneal cavity and grew as peritoneal implants, the perfusion patterns of these peritoneal metastases and their detectability with hypoxia imaging were compared with those of rats with orthotopic liver tumors. Four weeks after implantation, tumors reached the appropriate size for hypoxia analysis and imaging (5–20-mm diameter), and rats reached weights of 280–330g.

Direct in Vivo Oxygen Tension Measurements

In two of the tumor-bearing animals, direct measurements of oxygenation status in orthotopic liver tumors, peritoneal metastases, normal liver tissue, and normal muscle

Advances in Knowledge

- Iodine 124 (^{124}I)-labeled iodoazomycin galactopyranoside (IAZGP) uptake on PET images was found to be consistent with independent measures of hypoxia (direct oxygen tension probe measurements and the immunohistochemical hypoxia markers pimonidazole and EF5).
- PET imaging of tumor hypoxia with ^{124}I -IAZGP was found to be optimal 6 hours after tracer administration.

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Abbreviation:

IAZGP = iodoazomycin galactopyranoside

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Guarantor of integrity of entire study, C.C.R.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, C.C.R., P.B., P.B.Z., Y.S.C., Y.W., C.C.L.; experimental studies, C.C.R., P.B., P.B.Z., Y.S.C., Y.W., P.S., B.W., C.C.L., Y.F.; statistical analysis, C.C.R., P.B., S.C.; and manuscript editing, C.C.R., P.B., P.B.Z., Y.W., S.C., B.W., H.H., Y.F.

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tissue were performed (B.W.) with a fluorescence fiberoptic oxygen probe (OxyLite; Oxford Optronix, Oxford, England) (23). The experimental procedure has been described in detail elsewhere (11). Tissue oxygen tension was measured in three liver tumors with diameters of 0.7, 1.0, and 1.5 cm; in one peritoneal metastasis 1.1 cm in diameter; and in normal liver and muscle tissue. Measurements of both probes were performed twice simultaneously in two paths every 0.5 mm through the entire width of the tumor or at various depths in normal tissue. Four paths were measured per tumor.

Radiolabeling of the Compound

IAZGP was radiolabeled with ^{124}I , as previously described, by means of exchange labeling between the nonradioactive iodoazomycin nucleoside and ^{124}I -sodium iodine (Eastern Isotopes, Somerset, NJ). The final product was purified with anion exchange chromatography (21). Radiochemical purity, which was evaluated with thin-layer chromatography, was more than 99%.

Experimental Setup

Twenty-four hours before sacrifice, nine rats with one to three liver tumors each (16 tumors in total, 4–20 mm in diameter) and at most one intraperitoneal metastasis each (two metastases in total, 10 and 12 mm in diameter) were injected with 18.5 MBq (500 μCi) of ^{124}I -IAZGP, as well as with two well-established hypoxia markers—pimonidazole (60 mg per kilogram of body weight, HypoxyprobeTM-1; Chemicon International, Temecula, Calif) (24) and EF5 (30 mg/kg, C. Koch, University of Pennsylvania, Philadelphia, Pa)—via the tail vein (25,26). Two minutes prior to sacrifice, a 60 mg/kg dose of fluorescent dye (Hoechst 33342; Sigma, St Louis, Mo) was injected to enable depiction of the distribution of perfusion within the tumor. A second set of four rats, each with one or two liver tumors (15–20 mm in diameter), was injected intravenously with 18.5 MBq (500 μCi) of ^{124}I -IAZGP for serial PET imaging.

Hypoxia Imaging at a Microscopic Level

After animals in the first set were sacrificed, tumors were harvested en bloc with

their surrounding tissue, embedded in optimal cutting temperature cryofixative (Sakura Finetek, Torrance, Calif), and frozen in dry ice and isopentane. Frozen 10- μm -thick tissue sections were cut from various levels of the specimens and mounted on poly-L-lysine slides (Fischer Scientific, Pittsburgh, Pa). For high-spatial-resolution analysis of IAZGP distribution within tumors, slides were placed onto an imaging phosphor plate for autoradiography and stored at -20°C . Three days later, the images were read at 100- μm resolution by using a phosphor plate reader (model G-350; Bio-Rad Laboratories, Hercules, Calif). Subsequently, perfusion marker distribution was assessed on the identical tissue sections. For this assessment, up to 332 images of tumors up to 2 cm in diameter were acquired with a 4',6-diamidino-2-phenylindole fluorescent filter and 5 $\times$ magnification on a Zeiss Axiovert 200M inverted-stand microscope (Carl Zeiss, Oberkochen, Germany). The images were assembled into a montage by using Metamorph 6.2r3 software (Universal Imaging, Downingtown, Pa), and the color blue was assigned for Hoechst fluorescence visualization (C.C.R.).

Subsequently, slides were fixed with cold acetone, blocked with SuperBlock Blocking Buffer (Pierce, Rockville, Ill), in phosphate-buffered saline and stained with fluorescein isothiocyanate α -conjugated purified antipimonidazole antibody (Hypoxyprobe-1 Plus Kit; Chemicon International) and cyanine dye conjugated primary mouse anti-EF5 antibody (ELK3-51; C. Koch). Fluorescent microscope images were acquired by using fluorescein isothiocyanate and cyanine dye fluorescent filters, and the colors green and red were assigned for pimonidazole and EF5, respectively. The individual image frames were montaged to create one image of the entire tissue section. Finally, the slides were stained with hematoxylin-eosin, imaged under a bright field, and assembled into a montage again. To compare the distribution of the hypoxia markers and the tracer ^{124}I -IAZGP, images were overlaid by using the aforementioned Metamorph software.

Hypoxia Micro-PET Imaging

Animals were imaged in the prone position with R4 or Focus 120 micro-PET dedicated small-animal PET scanners

Figure 1: Overview of experimental setup.

(Concorde Microsystems, Knoxville, Tenn) with transaxial and axial fields of view of 10.0 cm and 7.8 cm, respectively. In the first set of nine rats, 12-hour PET scans were obtained 24 hours after tracer administration. In the second set of four rats, serial 20-minute PET scanning was performed 30 minutes and 1, 2, 3, 6, 9, 12, and 18 hours after tracer administration. Furthermore, two 1-hour PET scans were performed 24 and 48 hours after tracer administration. An energy window of 350–750 keV and a coincidence timing window of 6 nsec were used. The resulting list-mode data were sorted into two-dimensional histograms with use of Fourier rebinning, and transverse images were reconstructed in a $128 \times 128 \times 63$ matrix with the R4 scanner or a $128 \times 128 \times 96$ matrix with the Focus 120 scanner. The image data were corrected for nonuniformity of scanner response, dead time count losses, the I^{124} positron branching ratio, and physical decay to the time of injection. No cor-

rection was applied for attenuation, scatter, or partial-volume averaging. The PET counts in the reconstructed images were converted to activity concentration (measured in percentage of injected dose per gram of tissue) by using a system calibration factor (measured in microcuries per milliliter per counts per second per voxel) derived from imaging a rat-sized water-equivalent phantom region of interest. Analysis of the acquired images was performed with ASIPro software (Concorde Microsystems). Iodine uptake in the thyroid and stomach was not blocked.

To allow anatomic orientation of the PET images, computed tomographic (CT) scanning was performed by using a dedicated small-animal CT scanner (XSPECT; Gamma Medica, Northridge, Calif). Six hours before CT scanning, approximately 3 mL of the liver contrast agent Fenestra LC (Alerion Biomedical, San Diego, Calif), a suspension of iodinated chylomicron remnants, was admin-

istered intravenously. In contrast to the rapid acquisition times of clinical helical CT scanners, micro-CT scanners require approximately 10 minutes to acquire CT images, thereby rendering conventional water-soluble rapidly cleared contrast agents ineffective. The aforementioned liver contrast agent (Fenestra LC) is comprised of iodine-impregnated chylomicron particles that have apolipoprotein E on their surface. Such particles rapidly bind to and localize in hepatocytes via their apolipoprotein E receptors and remain localized within the normal liver for an extended period of time (up to ~1 day). Since cancer cells generally lack apolipoprotein E receptors and thus do not concentrate the aforementioned liver contrast agent, liver tumors appear as unenhanced foci on these contrast material-enhanced CT images.

Image Analysis

PET and CT image fusion and image analysis was performed by using ASIPro software (Concorde Microsystems). Coregistration of PET and CT images was performed manually and aided by fiducial markers placed on the animals prior to scanning. Two radiologists (C.C.R., P.B.) manually drew regions of interest over tumor, liver, and muscle tissue for each PET time point. For each tissue type and for each time after injection, the measured tissue activities were expressed as the mean $\pm$ standard deviation of the percentage of injected dose per gram of tissue, and tumor-to-liver and tumor-to-muscle ratios were derived. The mean tissue activities and the ratios were then plotted versus time. To verify region of interest measurements, selected tissues were harvested, weighed, and counted in a scintillation well counter calibrated for ^{124}I (1282 CompuGamma; LKB Wallac, Turku, Finland).

Statistical Methods

In vivo oxygen tension measurements in normal tissue and tumor tissue were compared by using a mixed-effects model, where tissue type was a fixed effect and animal and lesion type were random effects. Statistical software

Figure 2: Box-and-whisker plot of direct in vivo oxygen tension measurements in three liver tumors (*Liv Tu1*, *Liv Tu2*, *Liv Tu3*), one peritoneal metastasis (*Perit.met.*), normal liver tissue (*Liver*), and normal muscle tissue (*Muscle*) in two rats. The bold horizontal lines indicate the median value for each tumor or tissue, the boxes indicate the 25th–75th percentiles, and the whiskers indicate the entire range of measured values.

Figure 3: Matched set of data demonstrates association of various hypoxia markers, ^{124}I -IAZGP, and a perfusion marker. All staining was performed on a single 2-cm-diameter section of an orthotopic liver tumor. *A*, Autoradiogram obtained with ^{124}I -IAZGP. The light area corresponds to an area of high activity. Immunofluorescence staining with the hypoxia markers, *B*, EF5, and, *C*, pimonidazole. *D*, Tissue slice obtained for anatomic reference shows the normal liver (*L*), tumor (*T*), and necrotic areas (arrows). (Hematoxylin-eosin stain; original magnification, $\times 2$.) The black rectangle corresponds to white rectangle in *F* and the magnified view (*G*). *E*, Fluorescence staining with the perfusion marker. *F*, Overlay of *B*, *C*, and *E*, with the concordant areas of EF5 (green) and pimonidazole (red) staining appearing yellow. *G*, Magnified view of the area within the white rectangle in *F* and *D*. Note that the colocalized hypoxia markers EF5 and pimonidazole (yellow) typically are located a finite distance from the blood vessels (blue). This presumably reflects the effective diffusion distance of oxygen within the tumor.

(Proc Mixed, version 9.1; SAS Institute, Cary, NC) was used to fit this model. For analysis of the correlations among the various hypoxia and perfusion markers, a grid was placed in the identical position over the tumor tissue in each image, and signal intensities were measured by using the Metamorph software and compared on a pixel-by-pixel basis by computing Pearson correlation coefficients. In addition, region-of-interest image intensity measurements obtained with PET were correlated with scintillation counter measurements of tracer uptake with Pearson correlation coefficients.

Results

Oxygen Tension Measurements

The median oxygen tension of the various tumors ranged from 0.2 to 0.8 mm Hg, which was significantly lower ($P <$

.005, mixed-effects model) than the median oxygen tension in normal liver (45 mm Hg) and muscle (29 mm Hg) tissue (Fig 2). With the exception of sporadic outlying measurements of 10–80 mm Hg, which were possibly attributable to the proximity to blood vessels within the tumors, measurements were uniformly low throughout each tumor.

Hypoxia Imaging on a Microscopic Level

At visual inspection, high-uptake areas of the hypoxia tracer ^{124}I -IAZGP and the hypoxia markers pimonidazole and EF5 corresponded well with each other and with low-uptake areas of the perfusion marker (ie, reduced-blood-flow areas) (Fig 3). When image signal intensities were compared on a pixel-by-pixel basis, strong positive correlations were found among the hypoxia tracer ^{124}I -IAZGP and the hypoxia markers with a mean r value of 0.82 ± 0.06 (standard deviation) in all animals. Negative cor-

relations were found among the hypoxia tracer and the perfusion markers, with a mean r value of -0.44 ± 0.02 (Fig 4). On a microscopic level, the distribution of the hypoxia markers showed pronounced variations, with hypoxic regions typically located between blood vessels (Fig 3g). On a macroscopic level, that is, on low-power images of the tumor sections (Figs 3, A–C; Fig 5, C, D) and on micro-PET images (Fig 6), the distribution of the hypoxia tracer appeared homogeneous, except in necrotic regions, which showed little or no tracer uptake. Two animals had lesions smaller than 5 mm in diameter; the intensity of hypoxia marker staining and the amount of hypoxia tracer uptake in these small lesions appeared to be comparable to those in large lesions (1–2 cm in diameter) in these animals (Fig 5). The small number of such cases ($n = 2$) precluded statistical testing of this observation, however.

In all animals, peritoneal metastases and liver tumors showed the same distribution patterns both for hypoxia and for perfusion markers. Only the surrounding healthy tissue showed distinct differences. While liver tissue showed high blood perfusion (demonstrated by staining with the perfusion marker) and considerable hypoxia tracer background levels, normal soft tissue of the abdominal wall surrounding peritoneal metastases showed low blood perfusion and no hypoxia tracer uptake (Figs 3, 5).

In Vivo Hypoxia Micro-PET/CT Imaging and ^{124}I -IAZGP Time-Activity Curves

Despite high background signal intensity in the liver and intestines, PET images obtained 24 hours after administration of ^{124}I -IAZGP clearly showed hypoxic liver tumors and peritoneal metastases in seven of the nine animals in the first set (Fig 6).

During serial imaging in the second set of four animals, high background tracer levels in normal liver tissue precluded visualization of specific ^{124}I -IAZGP uptake in hypoxic tumor areas at early time points (ie, less than 2 hours after tracer administration) in all animals. At 3 hours after tracer administration, tumors were barely detectable; at 6 hours after tracer administration, tumors were depicted, as the liver background signal intensity had decreased sufficiently below the tumor signal intensity (Fig 6). The average percentage of injected dose per gram of tissue 6 hours after injection of ^{124}I -IAZGP was $0.13\% \pm 0.024$ in tumor tissue, $0.067\% \pm 0.010$ in liver tissue (tumor-to-liver ratio, 2.0), and $0.04\% \pm 0.04$ in muscle tissue (tumor-to-muscle ratio, 3.6). Imaging was possible for up to 48 hours after administration of ^{124}I -IAZGP; however, at this point, signal

intensity was progressively decreasing in all tissues. The average percentage of injected dose per gram of tissue 48 hours after injection was $0.033\% \pm 0.007$ in tumor tissue, $0.017\% \pm 0.06$ in liver tissue (tumor-to-liver ratio, 1.9), and $0.0095\% \pm 0.004$ in muscle tissue (tumor-to-muscle ratio, 3.5). There was a positive correlation ($r = 0.71$) between the signal intensities, as determined by using region-of-interest analysis of PET images, and tracer uptake, as determined by scintillation counting (Fig 7). Since high counts and thus low statistical uncertainty (ie, "noise"), as well as high tumor-to-background ratios, are preferred, the optimal time point for imaging hypoxia in rat liver tumors with ^{124}I -IAZGP PET was determined to be 6 hours.

High radioactivity was seen in the intestines over the entire 48-hour period, reflecting radioiodine release

Figure 4: Scattergrams show positive correlation between hypoxia markers and the hypoxia tracer (top row) and negative correlation between hypoxia markers, the hypoxia tracer, and the perfusion marker (bottom row). Measurements are based on pixel intensities of a grid placed over images shown in Figure 3, A–C, and E.

that resulted from catabolism of ^{124}I -IAZGP. This background signal intensity did not interfere with the depiction of tumors in our study; however, it could be reduced, if necessary, by coadministering sodium perchlorate, a competitive inhibitor of the sodium-iodide symporter.

Discussion

As new hypoxia-targeted treatment regimens enter into clinical trials, it will become important to noninvasively assess the presence of hypoxia and monitor changes in the levels of hypoxia during treatment. Although PET represents a promising technology for hypoxia imaging, to our knowledge, the optimal hypoxia tracer has yet to be determined. High nonspecific background activities of nitroimidazole tracers within the liver make imaging hypoxia in subdiaphragmatic tumors particularly challenging (21,22).

Zanzonico et al (21) showed it was possible to perform tumor hypoxia imaging with ^{124}I -IAZGP in a mouse flank tumor model and demonstrated the feasibility of acquiring PET images over a 48-hour period. They reported that tumors larger than 300 mg had a signal intensity that was 10 times higher than that of muscle and three times higher than that of the liver 24 hours after injection. In our orthotopic rat liver tumor model, maximal tumor-to-muscle and tumor-to-liver signal intensity ratios of only 5.0 and 2.0, respectively, were achieved. Despite the lower ratios, tumor hypoxia could be detected with PET imaging in most ($n = 11$) of the 13 animals studied. Laughlin et al (26) studied the biodistribution of the hypoxia marker EF5 labeled with carbon 14 in EMT6 tumor-bearing BALB/C mice and found that 30 minutes after injection, the EF5 levels in tumors were among the lowest EF5 levels in all body tissues, whereas 24 hours after injection, EF5 levels in tumors were significantly higher than EF5 levels in all other tissues except the esophagus (which had a similar EF5 level) and the liver (which had a twofold greater EF5 level). Initial PET imaging studies of ^{18}F EF5 in

Figure 5: Images acquired from cryosections of a liver tumor and a peritoneal metastasis. *A, C, and E* show a 1-cm-diameter liver tumor (LT) and the surrounding liver (L). *B, D, and F* show a 1-cm-diameter peritoneal metastasis (M) in the abdominal wall with a 1.5-mm-diameter satellite tumor (arrows). *A, B*, Histologic tissue specimens. (Hematoxylin-eosin stain; original magnification, $\times 2$.) *C, D*, Overlays of immunofluorescence staining with the perfusion marker (blue) and the hypoxia marker (red). *E, F*, Autoradiographs of the hypoxia tracer ^{124}I -IAZG. A similar pattern of hypoxia tracer uptake throughout the entire tumor was observed in both the small (1.5-mm) tumor and the larger (1-cm) tumor. ^{124}I -IAZGP liver background signal intensity was low in the connective tissue of the abdominal wall and high in the normal liver tissue.

Morris hepatoma tumor-bearing rats showed EF5 to be a promising hypoxia imaging agent when imaging was performed 120 minutes after injection; however, there were high background levels in the liver and abdomen (22). A recent study of ^{64}Cu diacetyl-bis(N^4 -methylthiosemicarbazone) PET imaging demonstrated prohibitively high liver background activity at imaging of liver tumor hypoxia 1 hour after injection

(17). Although biodistribution studies have shown a slow progressive decrease in liver activity, it is unclear if the 12.7-hour half-life of ^{64}Cu is sufficiently long for successful late imaging of liver tumor hypoxia.

The current study supplements a parallel study performed by our group in which we compared the pharmacokinetics of ^{124}I -IAZGP and ^{18}F -fluoromisonidazole (MISO) at dynamic micro-

Figure 6: PET, CT, and combined PET/CT scans of a rat in the prone position with two liver tumors (LT) and one peritoneal metastasis (M) show in vivo detectability of hypoxic tissue in orthotopic tumors. Axial (left column) and coronal (right column) images are shown. Top: CT images obtained with the liver contrast agent. Middle: Coregistered PET/CT images. L = liver, St = stomach.

Figure 7: Graph shows tissue activities and tumor-to-background ratios over time. At each time point, the plotted point represents the mean. Solid lines demonstrate tissue activities in the liver tumor (●), liver tissue (■), and muscle tissue (▲), as assessed with region-of-interest measurements on serial PET images. Dotted lines demonstrate tumor to liver (●) and tumor to muscle (▲) ratios. The accuracy of measurements was verified with scintillation counting of weighed tissue samples harvested 48 hours after tracer injection. For the sake of clarity, standard deviations for important time points are given in the text.

scopic PET of four rats (27). The design of the latter study allowed direct comparison of the two tracers in the same animals by means of injection of ^{18}F -MISO followed by injection of ^{124}I -IAZGP 24 hours later (27). The study actually showed that ^{18}F -MISO yielded superior diagnostic image quality in the orthotopic Morris hepatoma RH7777 tumor model, contrary to the expected finding of a higher hypoxia image contrast for ^{124}I -IAZGP than for ^{18}F -MISO, which was observed by Zanzonico et al (21) in the MCa tumor model. In the current study, we provided a more detailed analysis of the biodistribution of ^{124}I -IAZGP, identified the optimal time point for PET imaging of this tracer, and provided direct oxygen tension probe and histologic confirmation of the tracer localization in tumor hypoxia in the orthotopic Morris hepatoma RH7777 tumor model.

A common assumption regarding tumor hypoxia is that solid tumors consist of a hypoxic center surrounded by well-oxygenated cells in the periphery. In contrast, in our tumor model, microscopic hypoxic areas were present throughout the entire tumor up to the periphery (Figs 3, 6). While macroscopic heterogeneity of tumor hypoxia has been described in certain cell lines and cancer types (28), a homogeneous hypoxia distribution involving the entire tumor has also been described in a variety of cell lines in xenografts and in a variety of patient specimens, including liver tumors harvested at surgery (6,29,30).

Furthermore, contrary to another common assumption—that large tumors are more hypoxic than small tumors—no difference in the hypoxia pattern could be discerned between small and large liver tumors in our model. Thus, it appears hypoxia was present beyond a certain distance (on the order of 100–200 μm) from individual blood vessels (not beyond a certain depth into the tumor). This observation can be explained by the concept of the diffusion-limit distribution of oxygen from a functional blood vessel (31). As tumor cells near a vessel proliferate, they slowly push adjacent cells further away into

areas of low oxygen concentration and eventually into necrosis. The resulting gradient of oxygen tension, which decreases progressively with increasing distance from the vessels, can be observed in Figure 3, G.

In vivo depiction of tumors with PET and other radionuclide imaging techniques is, of course, complex and affected by many factors, such as the size, depth, tracer concentration, and object contrast (ie, lesion-to-background activity concentration ratio) of the tumor, as well as the spatial resolution and sensitivity of the imaging system. It is not surprising, therefore, that in some instances (as in two of the nine animals in our study) tumors may not be visualized with PET. For example, partial volume averaging may result in nonvisualization of smaller lesions within background tissue of high activity concentration (eg, within the liver in the case of ^{124}I -IAZGP PET).

It could be seen as a limitation of the current study that the radiation dose (18.5 MBq) administered to rats (body mass, ~250 g [0.25 kg]) was considerably greater, on a per kilogram of body weight basis (18.5 MBq/0.25 kg = 74 MBq/kg), than that which could reasonably be used in patients (74 MBq/kg $\times$ 70 kg = 5200 MBq, assuming a standard body mass of 70 kg). It is rather standard practice, however, to use high radiation doses in proof-of-principle animal imaging studies. For example, radiation doses of approximately 7.4 MBq (200 μCi) of ^{18}F FDG are commonly used in PET imaging studies of xenograft tumor models in mice. We project the use of only approximately 126 MBq (3.4 mCi) of ^{124}I -IAZGP for clinical imaging.

In our study, hypoxia in orthotopic rat liver tumors was successfully imaged with PET by using ^{124}I -IAZGP. The optimal balance between tumor-to-background ratio and image intensity occurred 6 hours after tracer administration. Autoradiographic analysis of the regions of high ^{124}I -IAZGP uptake were shown to correspond to regions of intense pimonidazole and EF5 immunohistochemical staining, demonstrating that this tracer colocalizes with markers

known to concentrate in tissue hypoxia. Ongoing comparative studies with other tracers should help determine which tracer to use with which disease site in human trials.

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