Molecular Imaging of Hypoxia

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A wide variety of imaging approaches have been developed in the past few decades for monitoring tumor oxygenation and hypoxia in vivo. In particular, nuclear medicine has seen the development of several radiolabeled hypoxia markers and is the preferred method for imaging of tumor hypoxia. Hypoxia imaging is increasingly being used in the clinical setting and is progressing from a mere detection method to application in individualization of chemoradiotherapy.

Key Words: hypoxia; PET; optical imaging; nitroimidazole

DOI: 10.2967/jnumed.110.075663

Since its first observation by Tomlinson and Gray in 1955, tumor hypoxia has become a central issue in cancer treatment. Hypoxia can occur when the oxygen requirements of a tumor exceed those that can be delivered by its vasculature. Structural abnormalities of microvessels and the limited diffusion distance (<70 μm) of O2 within the tumor create hypoxic regions with partial pressure of O2 (pO2) typically less than 10 mm Hg. A recent review (1) described the following characteristics, which are often present in tumors, as contributing to impaired oxygen transport and hypoxia: a relatively sparse arteriolar supply; a high O2 consumption rate; low vascular density; inefficient orientation of blood vessels; intense variations in red blood cell flux, resulting in regions of cycling hypoxia; a limited arteriolar supply, which can lead to pathologically low vascular pO2 in regions distant from the arteriolar source (longitudinal O2 gradient); sluggish blood flow due to stiffening of hypoxic red blood cells and increased blood viscosity; and large-diameter shunts that can divert blood away from the tumor bed, resulting in regions of low pO2.

Tumors often adapt to hypoxic environments by upregulation of the transcription factor hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimer protein composed of O2-sensitive HIF-1α and constitutively expressed HIF-1β subunits. When stabilized by hypoxic conditions, HIF-1 binds and transactivates several of the genes associated with enhanced glycolysis, angiogenesis, and promotion of cell survival under oxidative stress. These characteristics eventually confer tumors with higher degrees of invasiveness and resistance to chemoradiation therapy. Consequently, patients with hypoxic tumors often have a poor prognosis and decreased overall survival rate. Therefore, the ability to detect hypoxia within tumors has significant implications for cancer management and therapy. The current gold standard for in vivo measurement of tumor oxygenation is the Eppendorf needle electrode system, which allows for direct measurement of pO2 in tumors. However, it is an invasive procedure that often requires ultrasound-guided placement of the electrode, and its use is limited to easily accessible tumors.

Immunohistochemical methods are extensively used to detect and quantify hypoxia in patient biopsies. They are typically based on antibody binding to bioreductive nitroimidazole compounds that are systemically administered before the biopsy is performed (e.g., pimonidazole, 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5)) or to endogenous proteins that are overexpressed under hypoxic conditions (e.g., HIF-1α, carbonic anhydrase IX). The main limitations of these techniques are their invasiveness, limited sampling size, and the inability to perform repetitive measurements in a patient to monitor changes in oxygenation after therapies. Because of the heterogeneous and dynamic nature of hypoxia in tumors, noninvasive techniques that permit serial imaging of hypoxia could provide valuable information on disease status and for treatment planning. Although hypoxia also occurs in other pathologic conditions including myocardial ischemia and stroke, hypoxia imaging is much more advanced in oncology applications such as prediction of response to therapy and overall prognosis. Therefore, this review focuses on the potential and current status of monitoring oxygenation and hypoxia in tumors. Both optical and PET methods will be discussed.

OPTICAL METHODS OF MEASURING TUMOR OXYGENATION AND HYPOXIA

Optical spectroscopy enables quantification of intrinsic sources of optical absorption, scattering, and fluorescence in tissue. There are 2 major sources of contrast relevant to hypoxia: hemoglobin oxygen saturation (StO2) and fluorescence redox ratio. Hemoglobin is the dominant tissue absorber throughout the visible spectrum. The heme groups of hemoglobin bind oxygen, which changes their absorption spectrum. Quantification of absorption at several wavelengths enables determination of the fraction of heme that is bound to oxygen (%StO2). The fluorescence redox ratio quantifies the autofluorescence associated with the electron carriers reduced nicotinamide adenine dinucleotide and flavin adenine dinucleotide (and related derivatives such as reduced nicotinamide adenine dinucleotide phosphate). The former is fluorescent only in its reduced form, whereas the latter is fluorescent only when oxidized (2). The ratio of the autofluorescence contributed by these compounds has thus been used as a ratiometric indicator of tissue metabolic activity.
PET of Tumor Hypoxia

Detection of tumor hypoxia with radionuclides was first demonstrated in 1981 by autoradiography with $^{14}$C-misonidazole, which selectively bound to metabolizing hypoxic cells within the tumor (11). Subsequently, $^{18}$F-misonidazole was proposed as a PET tracer for noninvasive imaging of tumor hypoxia. Since then, several other tracers also have been evaluated for this purpose, including $^{18}$F-fluoroazoxymycin arabinoside ($^{18}$F-FAZA), Cu(II)-diacetyl-bis(N$^4$-methylthiosemicarbazone) (Cu-ATSM) labeled with $^{64m}$Cu, and $^{18}$F-EF5 (Table 1). Readers are referred to recent reviews by Mees et al. (12) and Krohn et al. (13) for detailed discussion of these tracers. With the exception of Cu-ATSM, these compounds are 2-nitroimidazoles, and their retention in hypoxic cells is based on their ability to undergo sequential reduction reactions yielding products that bind to macromolecules within the cell. An ideal tracer for tumor hypoxia imaging should be specific for hypoxia, and its uptake should reflect clinically relevant cellular $pO_2$ values (0–10 mm Hg) irrespective of the tumor type and grade. An ideal tracer should also allow for radiolabeling without significant loss of biologic properties, be lipophilic enough to have uniform tissue distribution but hydrophilic enough to have faster clearance from systemic circulation and nonhypoxic tissue, and rapidly accumulate in tumor to give high tumor-to-background ratios at early times after injection. Absolute uptake in the tumor should be high to minimize patient radiation dose, and the tracer should have high stability against non–hypoxia-dependent metabolism in vivo.

Although none of the currently available tracers have all the properties of an ideal hypoxia imaging agent, $^{18}$F-misonidazole remains the most extensively studied agent. The selection of which imaging agent to use depends largely on, for example, tumor type, ease of synthesis, and availability of radionuclide. Several studies have validated the use of $^{18}$F-misonidazole PET for quantification of hypoxia in a variety of tumors, including head and neck cancer, non–small cell lung cancer, soft-tissue sarcoma, renal cell carcinoma, breast cancer, and brain tumors (14). These studies have used an $^{18}$F-misonidazole tumor-to-blood activity ratio of at least 1.2–1.4 at 2–2.5 h as the cutoff for defining hypoxia. The ability of $^{18}$F-misonidazole PET to provide prognosis and predict response to treatment has also been validated. In some studies, repeated PET of patients receiving chemotherapy or radiation therapy showed a reduction in tumor hypoxic fraction after treatment. The Trans-Tasman Radiation Oncology Group Study reported that hypoxia PET can identify cancer patients who may benefit from hypoxia-specific cytotoxins such as tirapazamine (15). Head and neck cancer patients with...
hypoxic tumors treated with a non–tirapazamine-containing regimen showed a higher risk for locoregional failure than did patients receiving tirapazamine. Nehmeh et al. evaluated the reproducibility of \(^{18}\text{F}\)-misonidazole PET scans in head and neck cancer patients and showed that tracer intratumoral distribution may vary between 2 scans (16). This variation can primarily be attributed to the cycling of acute tumor hypoxia, which has important implications for the use of \(^{18}\text{F}\)-misonidazole PET as a basis for dose painting in intensity-modulated radiotherapy (Fig. 2).

\(^{18}\text{F}\)–fluorooazomycin arabinoside (FAZA) is a 2-nitroimidazole hypoxia imaging agent with the alkyl side chain in \(^{18}\text{F}\)-misonidazole replaced by a polar arabinose sugar in an attempt to increase the overall hydrophilicity of the compound. Accordingly, \(^{18}\text{F}\)-FAZA was cleared more quickly from blood and normal tissues in animal studies and provided higher tumor-to-muscle ratios than did \(^{18}\text{F}\)-misonidazole. Similar to \(^{18}\text{F}\)-misonidazole, \(^{18}\text{F}\)-FAZA was found to be useful for imaging hypoxia in various tumors (18). In patients with glioblastoma multiforme, \(^{18}\text{F}\)-FAZA yielded remarkably high tumor-to-background ratios due to selective and presumably hypoxia-specific uptake in tumor reflecting blood–brain barrier disruption. \(^{18}\text{F}\)-FAZA does not cross the intact blood–brain barrier because of its hydrophilic nature, resulting in minimal uptake in normal brain. Grosu et al. have shown that \(^{18}\text{F}\)-FAZA PET can be used to define the target volume for dose escalation in radiation treatment planning (19). On the basis of current clinical experience, \(^{18}\text{F}\)-FAZA shows considerable promise for imaging hypoxia; how-

TABLE 1

<table>
<thead>
<tr>
<th>Hypoxia tracer</th>
<th>First clinical use (year)</th>
<th>Tumor-to-background ratio</th>
<th>Time after injection (h)</th>
<th>Disadvantages</th>
<th>Tumor types studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{18}\text{F})-misonidazole</td>
<td>1992</td>
<td>0.88–5.85</td>
<td>2–3</td>
<td>Longer biologic half-life; longer imaging schedule; peripheral metabolism; slow clearance from nonhypoxic tissue; peripheral metabolism</td>
<td>HNC, NSCLC, renal cell carcinoma, soft-tissue sarcoma, breast cancer, brain tumor, HNC, NSCLC, SCLC, lymphoma, gliomas</td>
</tr>
<tr>
<td>(^{18}\text{F})-FAZA</td>
<td>2007</td>
<td>1.2–3.7; for gliomas, 1.9–15.6</td>
<td>2–3</td>
<td>Longer biologic half-life; longer imaging schedule; peripheral metabolism</td>
<td>HNC, NSCLC, cervical cancer, rectal cancer</td>
</tr>
<tr>
<td>(^{18}\text{F})-EF5</td>
<td>2008</td>
<td>1.15–4.07</td>
<td>3</td>
<td>Longer biologic half-life; longer imaging schedule; peripheral metabolism</td>
<td>HNC, NSCLC, cervical cancer, rectal cancer</td>
</tr>
<tr>
<td>(\text{^{60}Cu})-ATSM</td>
<td>2001 ((^{60}\text{Cu})-ATSM)</td>
<td>1.0–10.4</td>
<td>0.5–1</td>
<td>Low specificity in some tumor types</td>
<td>HNC, NSCLC, cervical cancer, rectal cancer</td>
</tr>
</tbody>
</table>

*Maximal tumor-to-blood activity or tumor-to-muscle activity or tumor-to-background activity ratios.

**Fig. 2.** (A) \(^{18}\text{F}\)-misonidazole (FMISO) PET scans obtained 3 d apart in patient with head and neck cancer show large variations in size and distribution of hypoxic regions between scans. Tumor volume was defined by viable tumor tissue that showed \(^{18}\text{F}\)-FDG uptake. (B) Intensity-modulated radiotherapy dose distributions in color-wash display of patient whose sequential \(^{18}\text{F}\)-misonidazole PET scans were similar. Hypoxic target volume was defined from first scan (outlined in red), and boost dose of 14 Gy was delivered to this zone in addition to 70-Gy prescription dose. (Reprinted with permission of (18,19).)
ever, a direct comparison between 18F-FAZA and 18F-misonidazole in patients has yet to be performed. 18F-FAZA, another 2-nitroimidazole being developed for hypoxia imaging, is a pentafluoro derivative of the hypoxic cell sensitizer etanidazole and is prepared by electrophilic addition of 18F-F2 to the terminal double bond of the precursor. In designing EF5, Koch et al. used an opposing strategy to that of 18F-FAZA. They chose to enhance the lipophilicity of the compound by introducing 5 fluorine atoms to the nitroimidazole side chain to increase its biologic half-life and stability in vivo. 18F-EF5 is the most lipophilic 2-nitroimidazole hypoxia imaging agent with the longest biologic half-life. It has a unique advantage: EF5 in nonradioactive form has been extensively studied as a marker for hypoxia using fluorescence immunohistochemistry, and high EF5 binding was found to predict patient outcome. Other advantages of 18F-EF5 are its uniform access to all tissues, including the brain, and its high in vivo stability. The first human study of 18F-EF5 demonstrated hypoxia-specific binding in head and neck cancer. Similar to 18F-misonidazole, a tumor-to-muscle threshold of 1.5 has been suggested to define clinically significant hypoxia with 18F-EF5 (20).

Cu-ATSM is a nonnitroimidazole compound that is most commonly labeled with 60Cu (half-life, 24.5 min) or 64Cu (half-life, 12.7 h) for PET of tumor hypoxia (21). In the presence of hypoxia, Cu-ATSM is trapped intracellularly in the 1 e− reduced form, Cu(I)-ATSM. It provides high tumor-to-background ratios separated by 1–9 d in cervical cancer patients showed similar patterns and magnitudes of uptake, indicating the reproducibility and, most likely, the chronic hypoxia–dependent uptake of Cu-ATSM. However, 64Cu-ATSM yielded better-quality images with slightly higher tumor-to-background ratios than did 60Cu-ATSM (23). All clinical studies conducted with Cu-ATSM so far have used images acquired at less than 1 h after injection for evaluation of tumor hypoxia. Evidence from experimental studies suggests that the hypoxia selectivity of Cu-ATSM varies among different tumor types and that its uptake in some tumor types at 1–2 h after administration may not truly reflect hypoxia (24).

CONCLUSION

At present, there is no consensus on the best radiotracer for imaging hypoxia with PET. 18F-misonidazole, 18F-FAZA, and Cu-ATSM have demonstrated potential value in clinical studies; however, head-to-head comparison of these agents is necessary to reveal the optimal agent for a given tumor type. Although the search for an “ideal” hypoxia imaging agent is likely to continue, current PET hypoxia imaging methods already show promise for selection of patients who are likely to benefit from hypoxia-directed treatment regimens such as intensity-modulated radiotherapy, hypoxic cytotoxins, or HIF-1 inhibitors.

ACKNOWLEDGMENTS

This work was supported by grants from Varian Medical Systems, the DOD (W81XWH-07-1-0355), and the NIH (CA42324 and CA40355).

REFERENCES


