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Review Article

Tumor Hypoxia Imaging Agents in Nuclear Medicine

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HIGHLIGHTS

- Tumor hypoxia results in angiogenesis, apoptosis, metastasis, tumor aggressiveness, and treatment failure.
- Nuclear imaging can provide information about tissue oxygen levels.
- 2-nitroimidazole compounds selectively accumulate in hypoxic cells.
- At present a few PET radiopharmaceuticals as hypoxia imaging agents are in clinical trial stages.

ABSTRACT

Hypoxia is due to imbalance in oxygen supply and oxygen demand compromising biological functions of cells. Since tumor hypoxia results in angiogenesis, apoptosis, metastasis, tumor aggressiveness and treatment failure, in vivo measurement is required. Nuclear imaging can provide information about tissue oxygen levels. 2-nitroimidazole containing compounds selectively accumulate in hypoxic cells. They have been radiolabeled with ^{18}F, $^{123/124}I$, and ^{99m}Tc and used in clinical trial stages using PET and SPECT techniques. $^{62/64}Cu$-ATSM is a non-imidazole imaging agent, which is trapped in hypoxic cells. There is a great interest in the development of ^{99m}Tc-labeled 2-nitroimidazole compounds. Though novel compounds based on molecular mechanisms of hypoxia would be developed in future.

Introduction

Hypoxia is defined as a condition in which the oxygen level in tissues is below physiologic levels, which is due to imbalance in oxygen supply and oxygen demand compromising biological functions of cells. Hypoxia is categorized as chronic (diffusion limited hypoxia) and acute (caused by blood clots or tumor cell aggregation). Oxygen diffuses through capillaries into intracellular environment. Insufficient supply of oxygen changes intracellular metabolism, which results in several biological consequences depending on levels of hypoxia (Span and Bussink, 2015).

Solid tumors frequently face hypoxia because of abnormal changes in structure and function of the tumor vasculature together with altered diffusion conditions inside the tumor. In response to hypoxia, cells try to adapt themselves by up-regulating the production of numerous proteins. Many of the adaptations are by means of transcription factor Hypoxia inducible factor 1 (HIF-1). HIF-1 induces the target genes and leads hypoxic cells to up-regulate more than 100 proteins to overcome hypoxia and survive. It is well documented that tumor hypoxia results in angiogenesis, apoptosis, metastasis, tumor aggressiveness, and treatment failure (chemo-resistance and radio-resistance) (Mees et al., 2009; Sun et al., 2011; Span and Bussink, 2015).

Hypoxia measurements

Since tumor hypoxia has a poor prognosis, in vivo measurement of tumor hypoxia is required for assessing

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the aggressiveness of tumor and predicting the outcome of therapy. A variety of invasive and non-invasive techniques have been introduced for direct or indirect tumor hypoxia measurement. The gold standard of direct measurement is polarographic needle, which is inserted directly into a tumor. There are some drawbacks applying such technique including being invasive, requiring CT or ultrasound-guided placement as well as accessible tumors. Although it has been used in human tumors and animal models, it has never been approved to be used in clinical setting (Zhang et al., 2000; Le and Courter, 2008; Krohn et al., 2008; Span and Bussink, 2015).

Other methods such as phosphorescence imaging, blood oxygen level dependent, ^{19}F magnetic resonance imaging, and electron paramagnetic resonance imaging have also been used for direct measurement of hypoxia (Le and Courter, 2008; Mason et al., 2010).

Indirect measurements utilize molecular reporters of oxygen, which form stable adducts with intracellular macromolecules. The adducts fail to be formed at higher oxygen level (Le and Courter, 2008; Sun et al., 2011). 2-nitroimidazoles such as misonidazole and pimonidazole meet these criteria and bind to intracellular macromolecules. Several studies have indicated that nitroimidazole containing compounds have longer retention times in hypoxic cells than in normal cells. They have been used with immunohistochemical staining methods for in vivo studies. Fluorinated (^{19}F) and radiolabeled 2-nitroimidazole compounds have also been used in ^{19}F-MRI (as contrast agents) and nuclear medicine (as noninvasive imaging of hypoxia), respectively (Sun et al., 2011). 2-nitroimidazoles diffuses into cells and are reduced by intracellular reductases. In the presence of oxygen, they are re-oxidized and clear from cell. However, in the absence of oxygen, further reduction occurs yielding radical anions and reactive alkylating amine derivatives, which covalently bind to thiol groups of intracellular proteins and trapped in the cell (Linder et al., 1994; Krohn et al., 2008; Mees et al., 2009).

Non-invasive imaging of tumor hypoxia in nuclear medicine

Measuring hypoxia in vivo is difficult. External imaging techniques such as nuclear imaging can provide information about tissue oxygen levels. Nuclear medicine utilizes radiolabeled compounds for molecular imaging using positron emission tomography (PET), single photon emission computed tomography (SPECT) or gamma scintigraphy. There is a great interest in nuclear medicine in developing imaging agents that localize in hypoxic cells, since hot-spot images are more sensitive than negative or cold-spot images. Thus 2-nitroimidazole compounds, which selectively retain in hypoxic cells, have been radiolabeled and used in nuclear medicine using PET and SPECT. The first successful radiopharmaceutical agent based on 2-nitroimidazole is ^{18}F-Fluoromisonidazole (^{18}FMISO) which provides clinical images not only from necrotic tumor tissues but also ischemic myocardium in patients and experimental animals. Although it has been widely used in several studies in humans and animals, it has not been approved for routine clinical application because of some limitations. Its relatively high lipophilicity leads to high nonspecific binding, long washout times, slow localization in hypoxic cells, and a number of radioactive metabolites (Mees et al., 2009). Newer derivatives of 2-nitroimidazole compounds have been developed and evaluated such as ^{18}F-Fluoroetanidazole (^{18}FETA), ^{18}F-Fluoroerythronitroimidazole (^{18}FETNIM), ^{18}F-Fluoroazomycin-arabinofuranoside (^{18}FAZA), and $^{123/124}$I-iodoazomycin-arabinoside ($^{123/124}$IAZA). The new compounds are more water soluble with better pharmacokinetic properties (rapid clearance from blood and normal cells) (Mees et al., 2009). They are still in clinical trial stages.

A novel PET radiotracer that localizes in hypoxic tissue is ^{62}Cu(II)-diacetyl-bis(N-mthylthiosemicarbazone) (^{62}Cu-ATSM), a non-imidazole imaging agent which is composed of a metal complex of radioactive copper with ATSM. Cu(II)-ATSM is a neutral lipophilic small molecule which diffuses easily from blood into cells (rapid blood clearance). It is reduced in cells by cellular reducing equivalents and trapped in the hypoxic cells. Several clinical studies have approved its selective behavior for tumor hypoxia imaging (Mees et al., 2009; Sun et al., 2011). Though more clinical studies are required.

For routine clinical SPECT imaging, ^{99m}Tc is the radionuclide of choice. This is because it has good radiation physical characteristics [IT, 140 keV (90%), 6 h] and is available through an inexpensive ^{99}Mo/^{99m}Tc generator. Furthermore, it is well developed and has varied chemistry (Schibli and Schubiger, 2002).

The localization of 2-nitroimidazole in hypoxic tissues led to the preparation of nitroimidazoles substituted with the neutral, lipophilic technetium (v) oxo propyleneamine oxime (PnAO) complexes. ^{99m}Tc-labeled compounds, ″BMS181321 and BRU59-21″ are 2-nitroimidazole coupled to PnAO-type chelators. BMS181321, the first ^{99m}Tc-labelled 2-nitroimidazole, and its structural isomer BRU59-21 were developed and studied for tumor hypoxia imaging (Linder et al., 1994; Zhang et al., 2000). They have shown selective accumulation of radioactivity in hypoxic tissues. Although ^{99m}Tc-labelled BMS181321 retains in hypoxic cells in vitro and localizes in animal tumor models, it has never been used in clinical setting because of its in vitro and in vivo instability, high blood levels and extensive hepatobiliary excretion related to the high octanol/water partition coefficient (P=40) (Sun et al., 2011).

BRU59-21 (P=12), a second generation analogue of

BMS181321, has more stability in vitro and more rapid clearance compared to BMS181321. In preclinical studies it has proven to be safe and suitable for tumor hypoxia imaging (Mees et al., 2009; Sun et al., 2011).

P value (octanol/water partition coefficient) of 2-nitroimidazoles plays a crucial role on pharmacokinetic and biodistribution of these compounds. Since modification of P in these compounds was limited, Zhang et al. coupled 2-nitroimidazole to peptidic N_3S chelators for ^{99m}Tc. In these compounds, the value of P depends to amino acids.

The study demonstrated that a peptidic chelator to 2-nitroimidazole did not prevent the nitro group from being reduced enzymatically (Zhang et al., 2000). Peptides offer advantages over antibodies or proteins such as ease of production, small size, better penetration into tissues, favorable pharmacokinetics, and fewer immunologic reactions (Aloj and Morelli, 2004). The importance of peptide radiopharmaceuticals has encouraged vast researchers to apply an easy, fast and efficient peptide radiolabeling methods. Regarding high potential of peptides in diagnosis and therapy, radiopeptides containing 2-nitroimidazoles seem to be appropriate candidates for tumor hypoxia imaging.

Conclusion

At present, a few PET radiopharmaceuticals as hypoxia imaging agents are in clinical trial stages; however, their use is limited because they are expensive and only available at specific nuclear medicine centers. This has led to development of ^{99m}Tc-labeled compounds which are less expensive and more convenient to use. Since 2-nitroimidazole compounds have shown selective accumulation in hypoxic cells in human and animal models, there is a great interest in the development of ^{99m}Tc-labeled 2-nitroimidazole compounds. Though novel compounds based on molecular mechanisms of hypoxia would be developed in future.

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