An overview of the developments and potential applications of ^{68}Ga-labelled PET/CT hypoxia imaging

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Abstract

Non-invasive imaging of hypoxia plays a role in monitoring the body's adaptive response or the development of pathology under hypoxic conditions. Various techniques to image hypoxia have been investigated with a shift towards the use of molecular imaging using PET/CT. The role of hypoxia-specific radiopharmaceuticals such as radiolabelled nitroimidazoles is well documented particularly in the oncologic setting. With the increasing utilisation of in-house labelling with a PET benchtop generator, such as the ^{68}Ge/^{68}Ga generator, the use of ^{68}Ga-labelled hypoxic radiopharmaceuticals in the clinical setting is developing. Since hypoxia plays a role in various pathologic states including infectious disease such as TB, there is a need to explore the potential application of ^{68}Ga-labelled hypoxia seeking radiopharmaceuticals beyond oncology. The purpose of this review is to describe the developments of ^{68}Ga-labelled hypoxic radiopharmaceuticals including the various chelators that have been investigated. Further, the role of hypoxia imaging in various pathologies is discussed with particular emphasis on the potential clinical applications of hypoxia PET/CT in TB.

Keywords: ^{68}Ga-hypoxia imaging; Tuberculosis; ^{68}Ga-nitroimidazole PET/CT; Novel applications

Introduction

Low oxygenation levels are a hallmark of numerous pathologic conditions [1]. Hypoxia impacts the development and treatment of various disease types [2] and the ability to identify hypoxia has implications in a wide range of medical settings [3]. Hypoxia is a term used to describe a state where insufficient oxygen supply is present in tissues or organs to meet the cellular metabolic demand [3, 4]. Biologic consequences of hypoxia may be adaptive or pathologic [5] and depend on duration and the needs of individual cells [3]. Adaptive responses to hypoxia include increased ventilation, cardiac output, blood vessel growth and circulating red blood cells [6]. The endothelial lining of blood vessels elicit proinflammatory features where permeability is increased and anticoagulant properties are reduced as an abrupt response to hypoxia [5]. The endothelial response to hypoxia can be protective due to adaptation or maladaptive and dysfunctional with subsequent damage to an organ or tissue. Regulation of responses to hypoxia by the body's oxygen sensing systems

includes numerous adaptations at cellular level such as the regulation of specific genes. Oxygen-dependent enzymes such as asparaginyl-hydroxylase and prolyl-hydroxylase play a role in regulating hypoxia-inducible factor (HIF) which is one of the transcription factors of particular interest in hypoxia. [5, 6]. HIFs lead to adaptations of vascular homeostasis by activating multiple target genes. HIFs bind to hypoxia response elements (HREs) which target the promoter regions of cell survival, anagenesis, glycolysis and invasion targets as illustrated in Fig. 1 [6]. Hypoxia inevitably leads to a cascade of biological processes in both normal as well as diseased tissues with the persistence of hypoxia-induced genes resulting in exacerbation of existing pathology [7].

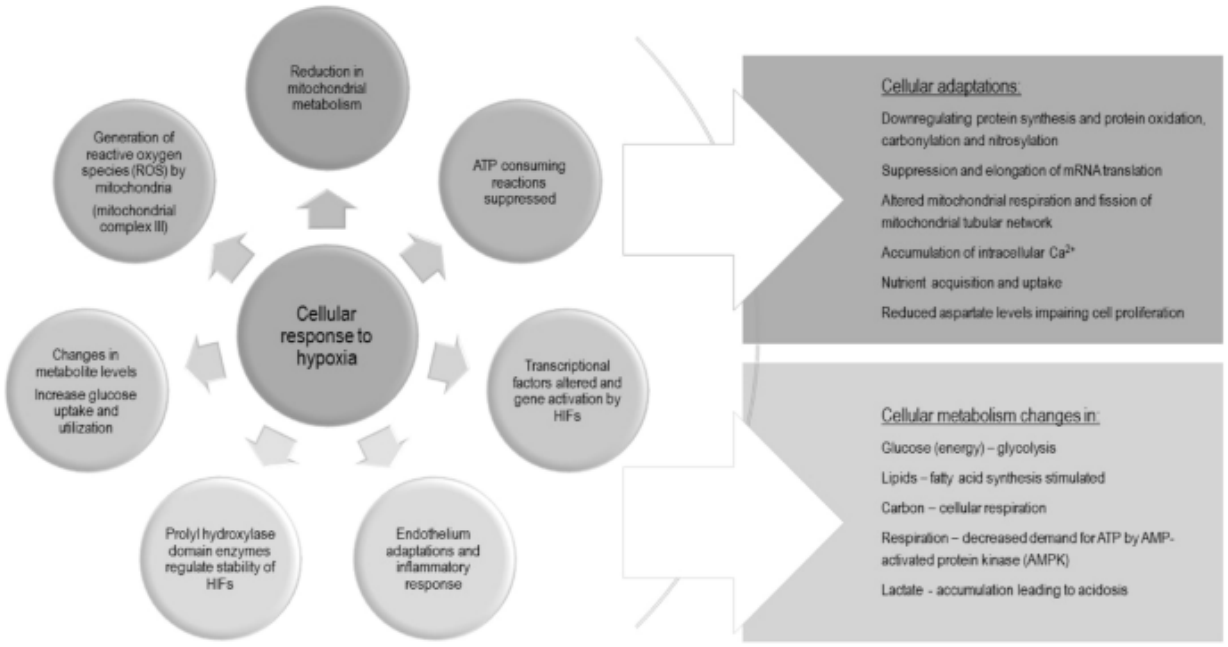

Figure 1. Cellular responses to hypoxia

Oxygen level or concentration (pO_2) can be measured or described in millimeters of mercury (mmHg) or as the oxygen percentage (%). Hypoxia can be described as mild (≤ 2% O_2/≤ 15.2 mmHg) or severe (< 1% O_2/< 7.6 mmHg) [7]. Oxygenation can be directly measured using invasive oxygen sensing tissue electrodes (Eppendorf probes) [7, 8]. The challenge with the use of tissue-based samples is the need for biopsy which can be considered as an invasive procedure that is dependent on the accessibility to the site for biopsy. Furthermore, oxygen-sensitive electrodes need to be properly calibrated, yield a small signal and require some form of imaging to assist with accurate placement [3]. This operator-dependent and technically demanding technique is, however, no longer commercially available [8].

Non-invasive methods for measuring levels of hypoxia include molecular imaging of tissue-based biomolecules based on the reducing nature of the hypoxic environment [7]. One such example is the exogenous bioreductive nitroimidazole drug [8], Pimonidazole, which has been used in various studies with particular emphasis on hypoxia in oncology [7]. Exogenous biomarkers have an oxygen sensing range of less than 1 mmHg and can be bound to fluorescent markers for detection by immunohistochemical methods [8]. Endogenous

biomarkers have an oxygen sensing range of less than 10 mmHg and provide information on the regional distribution of hypoxia in a tissue sample but this again requires invasive biopsy and the need for careful tissue sampling [8]. Bioluminescence and photo-acoustic imaging or optical-based approaches (phosphorescence and near-infrared spectroscopy) have excellent sensitivity [9] and are non-invasive techniques however, they are more prone to measure vascular pO_2 and not tissue pO_2 [4]. Optical imaging is generally two dimensional and with limited depth penetration [9].

Single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), near infra-red (NIR), optical imaging and positron emission computed tomography (PET/CT) are useful to detect hypoxia [10]. Technological advancements in radiological molecular imaging provide a safer alternative to evaluating hypoxia across a spectrum of diseases and applications. MRI may have some advantages over PET/CT as it yields high-resolution images [8] without imparting radiation to the patient [7]. To evaluate hypoxia, most MRI techniques use dynamic contrast-enhanced MRI (DCE-MRI) or blood oxygen level-dependent (BOLD) imaging [7]. There is concern that DCE-MRI provides indirect estimates of hypoxia since it measures perfusion [8] whereas hypoxia is influenced by other factors including haemoglobin saturation, vascular geometry oxidative phosphorylation. BOLD techniques in MRI measure differences in deoxygenated haemoglobin levels and have been used to map chronic hypoxic regions but do not correlate well with absolute pO_2 levels [7]. Other MRI techniques for hypoxia mapping that have been explored may be biased since the molecules explored distribute in the vasculature which impacts measurements of tissue oxygenation [4].

Mees et al. [11] present a discussion on the measurement of hypoxia using SPECT imaging. The first misonidazole derivative for hypoxia detection was labelled with the gamma emitting radionuclide Bromine-77. Iodinated versions of misonidazole have also been reported as iodovinyl derivatives [12] or iodinated sugars with attached nitroimidazoles such as iodo-azomycin arabinoside (IAZA) labelled with ^{123}I, ^{125}I or ^{131}I [11, 12]. The iodinated nitroimidazoles are lipophilic and demonstrate increased protein binding and slower blood clearance. A range of ^{99m}Tc-labelled hypoxia agents have been studied [11]. Despite possessing good SPECT imaging characteristics, these complexes are lipophilic, demonstrate slow reduction and instability in vivo and are retained in the cell due to lower permeability rather than irreversible binding and trapping [11, 12]. Although SPECT imaging is more frequently used than PET/CT, the superior resolution and more accurate quantification obtained through PET/CT makes PET/CT imaging of hypoxia preferable [11].

PET/CT can be considered as an ideal hypoxia imaging tool since the radiopharmaceuticals used are selective towards hypoxic tissue with high specificity despite the limited resolution of the images as compared to MRI and optical methods [4]. PET/CT thus allows for serial non-invasive assessment and mapping of hypoxia. Currently, hypoxia imaging is most frequently used to map hypoxia in tumours [13]. Application of PET/CT imaging for hypoxia is increasing due to the advantages of the imaging modality although its use in non-oncologic pathologies is an area requiring investigation.

PET/CT imaging of hypoxia

PET/CT offers good intrinsic resolution and provides options for quantification and semi-quantification of hypoxic burden. Three-dimensional representation of hypoxia can be mapped and fused for anatomic correlation with CT or MRI. Furthermore, a variety of radiopharmaceuticals and radionuclides are available to use for this patient friendly and non-invasive imaging modality. PET/CT also displays a high specificity for hypoxic tissue [9, 14], with the development and refinement of various hypoxia seeking radiopharmaceuticals. Compounds containing nitroimidazoles are among the most popular strategies used for tracking and imaging hypoxia [15]. The most frequently documented being the 2-nitroimidazole family of compounds labelled with Fluorine-18 (^{18}F). Hypoxia detection using PET/CT imaging has evolved with a shift towards complexing nitroimidazole derivatives with metal-containing radionuclides [2]. The purpose of this paper is to present the developments of Gallium-68-labelled (^{68}Ga) PET/CT hypoxia seeking radiopharmaceuticals and to propose its application beyond oncology.

Hypoxia-targeted radiopharmaceuticals

Compounds containing nitroimidazoles have the ability to be reduced and retained exclusively in hypoxic cells where they are irreversibly trapped [15]. The reduction of nitroimidazoles and subsequent uptake in hypoxic areas have previously been well described [3, 16]. Nitroimidazoles enter cells by passive diffusion [17] where they are reduced by intracellular reductases to intermediary metabolites. In the presence of oxygen, the molecules diffuse back out the cell after re-oxidation (Fig. 2). However, under hypoxic conditions, further reduction takes place, the metabolites covalently bind to thiol groups of intracellular proteins and the compound is trapped and accumulates in the cell [11, 18].

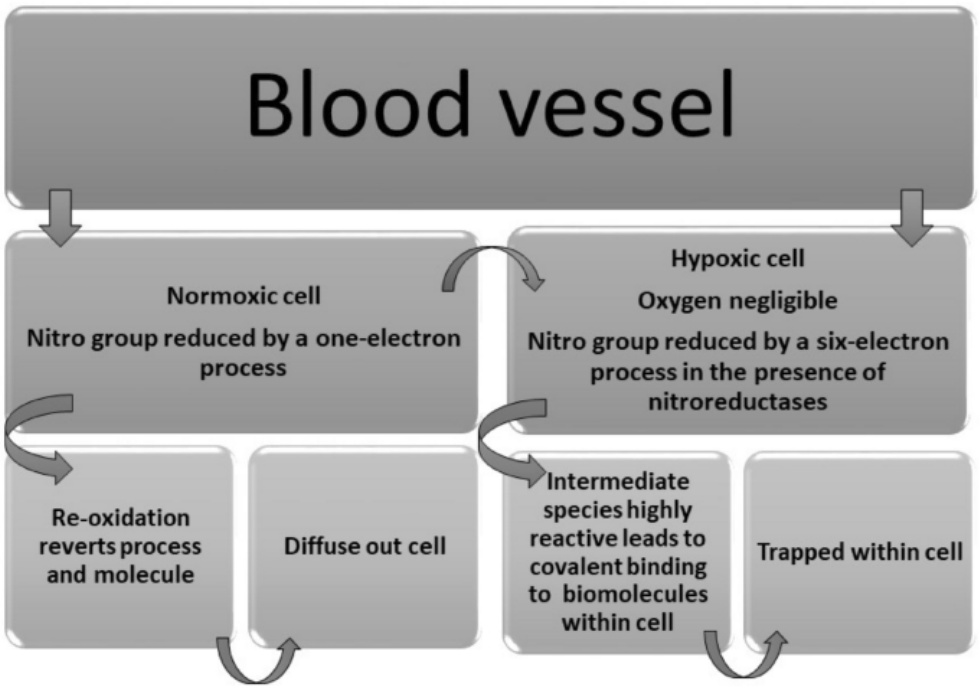

Figure 2. Reduction and trapping of radiolabelled nitroimidazoles

An ideal hypoxia-seeking radiopharmaceutical should be able to distinguish normoxia from hypoxia and should be able to image and possibly distinguish between acute and chronic hypoxia. As with all radiopharmaceutical preferences, the hypoxic agent should be simple, non-toxic, fast, easy to use and allow for repeated measurements. It should be lipophilic enough to allow homogenous distribution in tissue, while simultaneously being hydrophilic to enable fast elimination thus yielding high hypoxic to normoxic ratios. The radiopharmaceutical should not be degraded in vivo and should not depict aspecific tissue binding. The agent should be easy to synthesise, scalable and available in high purity with consistent batch reconstitutions [10]. Uptake of the hypoxic radiopharmaceutical should be dependent on the intracellular oxygen tension/hypoxic load rather than blood flow to the region. Finally the radiopharmaceutical and imaging should allow for quantification [11, 19]. Although most presently available PET/CT hypoxia imaging agents do not meet all the "ideal" hypoxia imaging characteristics, there are some derivatives that satisfy most criteria and are thus useful in clinical practice [20].

Currently, the nitroimidazole hypoxia imaging compounds available for PET/CT imaging include ^{18}F-fluoroerythronitroimidazole (^{18}F-FETNIM), ^{18}F-fluoroazomycin arabinoside (^{18}F-FAZA), ^{18}F-fluoroetanidazole (^{18}F-FETA), ^{18}F-flortanidazole (^{18}F-HX4) amongst others [3, 21, 22]. The development and use of the prototype and gold standard ^{18}F- fluoromisonidazole (^{18}F-FMISO) [3, 11, 22, 23] have been extensively studied and is still the most commonly used agent for hypoxia imaging [16, 21, 24]. The lipophilic nature of ^{18}F-FMISO leads to increased background tissue uptake and slow clearance from the body with the associated increase in radiation dose. Most hypoxic radiopharmaceuticals labelled with ^{18}F also suffer from lack of availability or complex labelling processes.

Other than nitroimidazole agents, an alternative based on a metal complex of radioactive copper has also been used to explore hypoxia in the myocardium. Copper-64-diacetyl-bis(N4-methylthiosemicarbazone) (^{64}Cu-ATSM) has shown selectivity for hypoxic tumours and ischaemic myocardial tissue although the mechanism of uptake is different to nitroimidazole uptake [3, 4, 16]. ^{64}Cu-ATSM clears rapidly from normoxic tissue [3]; however, ^{64}Cu production requires expensive target material thus limiting its use [25]. The hypoxic agent iodoazomycin arabinoside (IAZA) labelled with iodine-124 (^{124}I) has also proved to be promising preclinically in tumour cells [4]. ^{124}I-IAZA could not outperform ^{18}F-FAZA or ^{18}F-FMISO in terms of favourable biokinetics or tumour-to-normal tissue ratios [11, 26].

Advantages of ^{68}Ga-labelled radiopharmaceuticals

The development and refinement of bench-top Gallium-68 (^{68}Ga) generators allows for the convenience of in-house and economical labelling of PET/CT radiopharmaceuticals [27]. This presents a significant advantage compared to the cyclotron products of ^{18}F, ^{64}Cu and ^{124}I that require expensive, difficult chemical processing [25]. ^{68}Ga is a positron emitter with a half-life of 67.6 min. The 270.95-day half-life of the parent nuclide Germanium-68 (^{68}Ge) allows for one generator to be used for up to 1 year and provides two or three elutions per day. Thus, it is very cost-effective and obviates the need for an on-site cyclotron [15]. Many ^{68}Ga-labelled radiopharmaceuticals have been designed with the new molecules being based on the homology of the kit based ^{99m}Tc radiopharmaceuticals [28].

Table 1 Development of ^{68}Ga-hypoxia imaging agents

^{68}Ga-nitroimidazole hypoxia derivative	Tissue/cell line	Quantificat-ion	Primary bio-distribut-ion/excretion	Comments/findings	References
^{68}Ga-NOTA-NI (^{68}Ga-3) ^{68}Ga- SCN-NOTA-NI (^{68}Ga-4)	Chinese hamster ovarian cancer cell line (CHO) Murine colon cancer cell line (CT-26) cell lines Biodistribution studies in CT-26 xenografted mice	Tumour to muscle ratios Tumour to blood ratios	Kidneys Bladder Liver Intestine	Hypoxic uptake significantly higher than normoxic conditions at 1 h Tumour:blood ratios less than ^{18}F-FAZA and ^{18}F-FMISO at 1 h Tumour:muscle ratios comparable to ^{18}F-FAZA and ^{18}F-FMISO at 1 h ^{68}Ga-NOTA-NI showed lower intestinal uptake and higher tumour uptake than ^{68}Ga-NOTA-SCN-NI	[25]
^{68}Ga-DOTA-2-(2-Nitroimidazolyl) ethylamine (^{68}Ga-4) 6^{8}Ga-DOTA-2-(2-Nitroimidazolyl) ethylamine-SCN-Bz (^{68}Ga -5)	Henrietta lacks cervical cancer (Hela) Chinese hamster ovarian cancer cell line (CHO) Murine colon cancer cell line (CT-26) cell lines Biodistribution studies is CT-26 xenografted mice	Tumour to muscle ratios Tumour to blood ratios	Kidneys Bladder Liver Intestine	Hypoxic uptake significantly higher than normoxic conditions at 1 h. Initial tumour uptake decreased after 10 min Increased tumour to muscle and tumour to blood ratios due to rapid blood and muscle clearance ^{68}Ga-4 showed more distinct tumour uptake	[29]
^{68}Ga-DOTA-Nit1* ^{68}Ga-DOTA-Nit 2**	HCT-15 (corresponding to human colon adenocarcinoma) Biodistribution studies in 3LL Lewis murine lung carcinoma cells injected subcutaneously in the right limb of C57BL/6 mice	Tumour to muscle ratios Tumour to blood ratios	Low blood and liver activity. Primary excretion via urinary tract	Increased hydrophilicty and preferable biodistribution compared to ^{18}F-FMISO Moderate uptake of both agents in tumour at 30 min PI. Nearly 100% of ^{68}Ga-DOTA-Nit 2 taken up in tumour areas retained at 2 h. ^{18}F-FMISO still demonstrated better cell penetration and subsequent uptake at all time points. However, ^{68}Ga complexes showed better tumour to muscle ratios at all time points. Rapid soft tissue clearance of ^{68}Ga complexes	[23]
^{68}Ga-DOTA-MN2 (^{68}Ga-DOTA-metronidazole-2)	NFSa mouse fibrosarcoma (FM3A cells) inoculated in right thigh or right flank of 5-week-old female C3H/He mice	Tumour to muscle ratios Tumour to blood ratios	Kidneys and bladder	Clear visualisation of hypoxic uptake after 1 h. Superior to ^{18}F-FMISO and ^{18}F-FAZA for abdominal hypoxic imaging. More studies needed for progression toward clinical application (beyond oncology)	[31]

Table 1 (continued)

^{68}Ga-nitroimidazole hypoxia derivative	Tissue/cell line	Quantificat-ion	Primary bio-distribut-ion/excretion	Comments/findings	References
^{68}Ga-HP-DO3A-nitroimidazole (^{68}Ga-HP-DO3A-NI)	A549 lung cancer cells SCID mice bearing subcutaneous A549 tumor xenografts	Tumour to muscle ratios	Kidneys and bladder	Significantly more uptake in hypoxic cells in vitro and tumours in vivo. Compared to other ^{68}Ga-hypoxia agents there was less non-specific tissue uptake particularly the liver with better image contrast in tumours. No accumulation in heart, liver or lung	[32]
^{68}Ga-H2CHXdedpa (CHX = cyclohexyl/cyclohexane) (N4O2)-NI (^{68}Ga-22,-23,-24,or-30)	HT-29 (colon), LCC6HER-2 (breast), and CHO (Chinese hamster ovarian) cell lines	Hypoxic to normoxic ratios	No biodistribution study included	Uptake under hypoxic conditions for all cell lines. Largest increase in uptake between 30 and 60 min. Hypoxic:normoxic ratios highest at 60 min. Ideal candidates for in vivo testing	[15]
^{68}Ga-1,4,7-triazacyclononane-1,4,7-tris[methyl(2-car-boxyethyl) phosphinic acid] (TRAP)-nitroimidazole derivatives[#]	U87MG (human glioblastoma) and CT-26 (mouse colon cancer) cell lines, mouse colon cancer CT-26 xenografted BALB/c mice	Hypoxic to normoxic ratios Tumor to blood and tumor to muscle ratios	Kidneys and bladder	Lower protein binding than agents using NOTA and DOTA conjugates. Highest uptake and SUV in tumour cells at 1 h. ^{68}Ga-5 and ^{68}Ga-6 showed hypoxic uptake with ^{68}Ga-6 hypoxic:normoxic uptake ratios greater than NOTA and DOTA derivatives Trivalent agent showed the highest tumor uptake in biodistribution and animal PET studies	[30]
^{68}Ga-4- compound (Gallium(III) chloride bis(4-allyl-3-thiosemicarbazone) ace-naphthenequinone) (^{68}Ga-BTSC)	EMT6 cells Nude athymic mice	None	Kidneys and bladder	53% higher retention in hypoxic conditions in cells at 2 h. Slight accumulation in the liver	[2]

*^{68}Ga-DOTA-(10-[2-(2-methyl-5-nitro-1 H-imidazole-1-yl)ethylaminocarbonylmethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid)

**^{68}Ga-DOTA-Nit 2 (10-[N-methyl-1-[1-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)ethyl)-1 H-1,2,3-triazole-4yl]methylaminocarbonyl-methyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid)

[#]Derivatives ^{68}Ga-TRAP-3, ^{68}Ga-TRAP-4, ^{68}Ga-TRAP-5, ^{68}Ga-TRAP-6

^{68}Ga-labelled nitroimidazole derivatives have been synthesized and pre-clinically validated as promising candidates for hypoxia imaging [15, 22, 23, 25, 29,30,31,32]. Thus far ^{68}Ga-labelled nitroimidazoles have demonstrated greater hydrophilicity with faster clearance and increased target to background ratios as compared to the ^{18}F-labelled nitroimidazole counterparts [23, 31]. These characteristics could make the ^{68}Ga-labelled nitroimidazole derivatives ideal for hypoxia-specific PET/CT imaging in the clinic. Despite the preferable pharmacokinetics, the hydrophilicity of ^{68}Ga-labelled hypoxia radiopharmaceuticals may cause concern that not enough tracer will accumulate in hypoxic cells for detection [32]. However, the convenient labelling procedure and availability of an in-house ^{68}Ge/^{68}Ga generator together with the potential for diverse application of ^{68}Ga-labelled hypoxia imaging agents outweighs this concern. Some developments in ^{68}Ga-labelled hypoxia agents are summarised in Table 1. Pre-clinically, the most promising ^{68}Ga-labelled hypoxic agents demonstrated high hydrophilicity and superior uptake when compared to ^{18}F-FMISO and ^{18}F-FAZA. There was less liver, abdominal uptake and non-specific tissue uptake which would be ideal in human studies; however, this would need to be validated by robust clinical trials [30,31,32].

Chelators

Bifunctional chelating agents are commonly used in ^{68}Ga-labelling since they form highly stable radiometal chelates. Tsionou et al. [33] state that ideal chelators will “rapidly, quantitatively and stably coordinate $^{68}Ga^{3+}$ at room temperature”. A range of acyclic and macrocyclic chelators are available for reaction at variable temperatures and pH. Chelators that can be used at near neutral pH and low chelator concentration, achieving high radiochemical yield allow for simple routine and reproducible radiopharmaceutical formulation [33].

Hoigebezar et al. [25] synthesized nitroimidazole derivatives conjugated with two different bifunctional chelating agents. Both derivatives were stable at room temperature up to 4 h, showed low protein binding and labelled with high efficiency (96%). Hypoxic conditions resulted in increased uptake in cell lines. Tumour to muscle ratios calculated from animal PET imaging of CT-26 xenografted mice demonstrated that the more hydrophilic agent had increased tumour uptake. Uptake of both agents in cell lines was comparable to ^{18}F-FAZA and ^{18}F-FMISO [25]. A different chelator, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), was then experimented with since it is more hydrophilic than its corresponding 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) derivative [29]. Various ^{68}Ga-labelled agents are chelated using NOTA due to the high stability of the compound formed [34]. Labelling efficiencies were > 98% for the two stable, hydrophilic ^{68}Ga-derivatives that were labelled within 10 min. Rapid decreases in blood and muscle activity resulted in increased tumour to muscle and tumour to blood ratios over 2 h post injection [29].

In a separate study, Fernández et al. [23] synthesized two (Nit1 and Nit2) 5-nitroimidazole derivatives conjugated to ^{68}Ga with DOTA to evaluate their potential as hypoxia targeting agents with similar findings. The agents were synthesized within 15 min and had a radiochemical purity above 90%. Both agents were stable for at least 4 h in the labelling milieu, while there was 90% stability in human plasma after 2 h [23]. ^{68}Ga–NOTA–Nit1 and

^{68}Ga–NOTA–Nit2 showed low protein binding and were, therefore, more hydrophilic than ^{18}F-FMISO demonstrating a clear advantage over the slow washout of ^{18}F-FMISO from normoxic tissues. Both agents demonstrated preferential hypoxia uptake with ^{68}Ga–NOTA–Nit2 having similar hypoxia/normoxia ratios to ^{18}F-FMISO in cell culture. However, the ^{68}Ga–NOTA–Nit1 complex had a significantly higher ratio as compared to ^{68}Ga–NOTA–Nit2 and the ^{18}F-FMISO control. In vivo animal studies demonstrated moderate uptake of both agents at tumour sites with almost 100% of the dose taken up being retained after 2 h for the ^{68}Ga–NOTA–Nit2 complex. The ^{68}Ga-labelled complexes showed improved tissue clearance with higher tumour to muscle ratios than ^{18}F-FMISO. A potential drawback may be that the tumour activity was not found to be statistically higher than blood activity at any time point and therefore acts similar to ^{18}F-FMISO. Overall, the agents showed potential to replace ^{18}F-FMISO imaging [23]. In another animal study, tumour lesions were successfully imaged using PET/CT after the intravenous administration of radiolabelled metronidazole (^{68}Ga–DOTA–MN2). Similar to other studies [23, 25], moderate uptake of the ^{68}Ga-hypoxic agent with rapid clearance led to high tumour to non-target ratios as compared to ^{18}F-FAZA. Due to the lower background activity from the abdomen, the authors suggest ^{68}Ga–DOTA–MN2 would be superior to ^{18}F-FMISO and ^{18}F-FAZA for hypoxic imaging [31]. Similar biodistribution and hypoxic uptake was demonstrated by Wu et al. [32] who used a different ligand to the previous studies discussed. In vivo studies demonstrated peak uptake in hypoxic tumour areas within 10 min post-injection with rapid clearance from muscle. Subsequently, there was a linear increase in the tumour to muscle ratio over time. Therefore, the ^{68}Ga–HP–DO3A–NI derivative offers another attractive alternative to image hypoxia.

A group that investigated synthesis of mono- and bis(thiosemicarbazones) (BTSC) via microwave-assisted heating found that the BTSC structure required modification for the incorporation of gallium to ensure higher kinetic stability and limit dissociation after radiolabelling [2]. This group also found that ^{68}Ga–BTSC complexes are able to pass inside the cell, leading to dissociation upon interacting with hypoxic microenvironments in a similar fashion to ^{64}Cu-ATSM.

Ramogida et al. [15] investigated the application of four ^{68}Ga nitroimidazole derivatives using a linear cyclic hexadentate (N_4O_2) chelating agent, H2dedpa (1,2-[[6-carboxypyridin-2-yl]methylamino]-ethane) and its chiral derivative H2CHXdedpa (CHX = cyclohexyl/cyclohexane) (N_4O_2). Both chelators showed high thermodynamic stability and in vitro kinetic inertness. All derivatives showed uptake under hypoxic conditions with results comparable to in vitro ^{18}F-FMISO experiments, suggesting that the nitroimidazole derivatives would be ideal for further in vivo testing [15]. In another study, a different chelating agent 1,4,7-triazacyclononane-1,4,7-*tris*[methyl(2-car-boxyethyl)phosphinic acid] (TRAP), was used to bind mono-, bis-, and trisnitroimidazole conjugates to ^{68}Ga with 96% radiolabelling yields that were stable up to 4 h in human serum [30]. The trivalent agent showed the highest uptake in cell culture and in animal PET studies at 60 min post-injection. Similar radiochemical yields were obtained by Seelam et al. [30] who experimented with a series of hydrophilic TRAP-based nitroimidazole derivatives to overcome some of the limitations of ^{18}F-FMISO. They found that TRAP based derivatives were also stable up to 4 h in vitro and were more hydrophilic than NOTA- and DOTA-nitroimidazole derivatives at 60 min. Therefore, the agents could have the potential for rapid uptake and fast clearance from blood and non-target organs [30]. For preparation of ^{68}Ga-labeled

radiopharmaceuticals, NOTA is one of the most commonly used bifunctional chelating agents due to formation of stable compounds and a better selectivity towards the Ga^{3+} ion [35]. NOTA-nitroimidazole derivatives have demonstrated comparable uptake to ^{18}F-FMISO and ^{18}F-FAZA whereas DOTA derivatives yield more rapid muscle and blood clearance with less liver and abdominal uptake. We, however, recommend that TRAP be used as a chelator for ^{68}Ga-hypoxia labelling due to the increased hydrophilicity and since synthesis of TRAP as a chelating ligand is simple, fast and scalable [30].

Potential application of ^{68}Ga-hypoxia imaging

Identifying hypoxic tissue has therapeutic implications for multiple non-oncology-related disease states including stroke, myocardial ischemia, diabetes [3] and likely for infectious diseases.

PET/CT imaging of stroke has normally involved the use of perfusion radiopharmaceuticals such as Oxygen-15-based tracers. However, the limitations in the very short half-life and requirement for an onsite cyclotron limit the use in the clinical setting [36]. Experimental studies support the notion that nitroimidazoles can specifically detect viable but severely hypoxic tissue in acute stroke. However, the mildly or severely hypoperfused necrotic areas were not detected. This implies that hypoxia imaging in acute stroke can be used as a specific marker for the salvageable tissue [37] and can be used to predict clinical outcome [36]. Additionally, hypoxic imaging in stroke can enable the selection of patients that would benefit from a hypoxia-directed treatment [36]. Obesity and diabetes are associated with oxidative stress and inflammation within a chronically hypoxic environment. Renal hypoxia has been postulated to be the mechanism for chronic kidney disease associated with diabetic nephropathy [38]. Hypoxia also leads to neovascularization that is the primary cause of visual loss in diabetic retinopathy [39]. Hypoxia can lead to increased cell necrosis and apoptosis. Therefore, monitoring levels of hypoxia in such circumstances would be beneficial to inform and manage interventions [40].

The role of hypoxia in tumours has been well documented [11]. Approximately 60% of solid tumours exhibit areas of hypoxia or anoxia [4, 10]. Local hypoxia in malignancies results in resistance to radiotherapy [32] and chemotherapy [41] and is associated with tumour aggressiveness, reduced tumour control, increased recurrence and poorer prognosis [4, 41]. Wu et al. [32] suggest that non-invasive evaluation of tumour hypoxia would be valuable for screening cancer patients prior to radiation therapy. A factor to consider in tumour imaging is that the blood activity was always higher than tumour uptake in animal studies due to lower blood flow in the hypoxic than in the normoxic tumor [30]. This, however, may not be the case in other diseases where hypoxia plays a role.

The concept that hypoxia induces inflammation is well accepted. Hypoxia-induced inflammation is clinically relevant in organ grafts, acute lung injury, inflammatory bowel disease and infective processes [42]. The use of PET/CT in infection imaging is evolving since it enables the dynamic assessment of infectious processes and obviates the need for invasive tissue sampling. Infectious diseases are a worldwide health problem with tuberculosis (TB), human immunodeficiency virus (HIV) and malaria posing a significant burden to developing countries [43]. Nitroimidazoles can target a broad range of parasitic

and bacterial pathogens. Nitroimidazoles, such as metronidazole are still considered the treatment of choice for anaerobic infections [43] which may extend the use of the "hypoxic" PET/CT radiopharmaceuticals for use in detecting these pathogens [43]. Research has been undertaken to study the TB micro-environment in a pursuit to develop non-invasive molecular probes to effectively image TB and predict treatment outcome at an early stage [44,45,46]. Tuberculous granulomas in guinea pigs, rabbits and non-human primates are hypoxic and the hypoxic microenvironment is an important feature of TB lesions [47]. Belton et al. [48] found that TB lesions in humans are severely hypoxic. Hypoxia leads to a reduced metabolic state/latency and dormant disease and is one of the main processes underlying MDR-TB and LTBI pathology [46, 49, 50]. The core of most mature TB granulomas are necrotic and hypoxic (Fig. 3) where hypoxia is one of the factors playing a role in switching metabolism in TB to an inactive state promoting nonreplicating persistent (NRP) bacteria. Necrotic and hypoxic lung granulomas can significantly abate drug efficacy [50] since the NRP bacteria are largely resistant to many known antimicrobials [51].

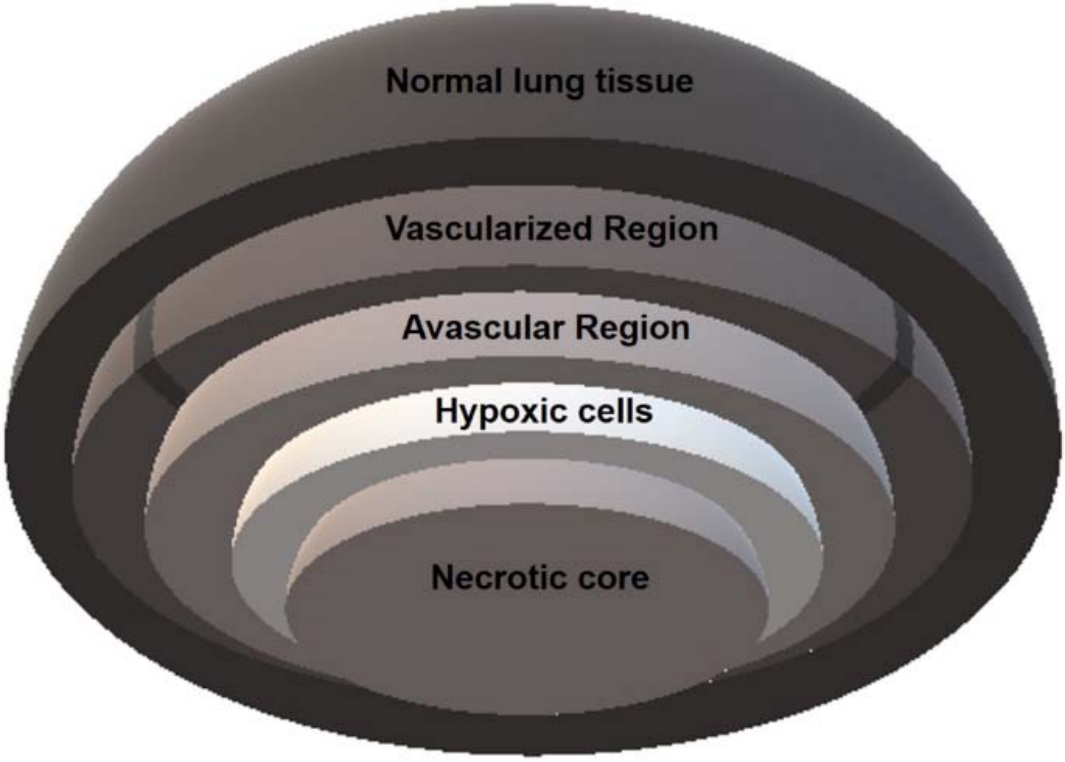

Figure 3. TB granuloma regions include hypoxic cells surrounding the necrotic core

Dormant TB bacilli are harboured within hypoxic environments of caseating granulomas. The dormant state is characterised by reduced replication and metabolism where stable latent bacilli survive under stressful conditions [51]. Oxygen tension is, therefore, associated with the outcome of TB infection [52]. Failure to control mycobacterial proliferation within granulomas can influence disease progressions and clinical outcome [53] since NRP bacteria can remain viable for prolonged periods and can become tolerant to many first line antibiotics [54, 55]. NRP bacteria, therefore, play a role in the long treatment duration of TB drug regimens [54]. If oxygen content drops (below 1%), there appears to be reduced susceptibility to standard TB drugs but increased susceptibility to nitroimidazole drugs.

Studies to assess shorter TB treatment regimens are ongoing including a nitroimidazole agent [56]. One would thus expect uptake of radiolabelled nitroimidazoles in hypoxic regions of TB lesions. Metronidazole is a nitroimidazole antibiotic medication used to treat anaerobic bacteria and protozoa. Pre-clinically, the radiolabelled derivative of metronidazole demonstrated a tendency to accumulate in hypoxic regions with uptake corresponding to of the exogenous hypoxic marker, pimonidazole [31].

Hypoxic PET/CT radiopharmaceuticals have already been validated for the management of cancer and could therefore play a role in the management of TB [21]. ^{18}F-FMISO has successfully been used to demonstrate heterogeneous uptake in hypoxic TB lesions within the same patient suggesting that hypoxia-specific radiopharmaceuticals can help understand TB pathogenesis [48]. Furthermore, the development of therapeutic interventions could be explored further by identifying susceptible lung tissue before irreversible damage occurs [46]. Much research has been done on hypoxia-specific radiopharmaceuticals; however, the focus has been primarily on PET/CT imaging of tumour hypoxia [4, 20]. Limited literature on the use of ^{68}Ga-labelled nitroimidazole derivatives in assessing hypoxia in infection and inflammation could be found although the concept of PET/CT hypoxic imaging in communicable diseases such as TB has been postulated [21, 49].

Imaging hypoxia has potentially significant roles in identifying and selecting patients who may respond better to treatments designed to overcome the limitations of hypoxia in disease. Serial imaging of hypoxia can thus be used to monitor and adapt the treatment strategy based on hypoxic load [3]. Although the study by Sano et al. [31] investigated hypoxia in tumour cells using radiolabelled metronidazole, this application could be extended to other diseases such as TB. Metronidazole has been recommended as a therapeutic option against Gram-negative and Gram-positive bacteria [57]. Metronidazole is important in the treatment of anaerobic infections and has proven effective in the treatment of mixed aerobic and anaerobic infections. Since hypoxia plays a role in TB, metronidazole or other nitroimidazoles could be considered as imaging and treatment options in an attempt to target the hypoxia within granulomas [43, 48]. Therefore, use of radiolabelled nitroimidazoles to image hypoxic load in TB would be useful considering the unique insight and understanding we could gain from such research [21]. Hypoxic imaging in TB could provide useful information for the latent stage of TB as well as evaluating risk of disease reactivation based on the presence of dormant bacilli within hypoxic regions [21]. Since the majority of TB therapies target actively replicating bacilli [58], PET/CT hypoxia imaging would allow for the identification of patients who would benefit from adjunct therapy that targets the anaerobic regions to selectively deplete the NRP bacteria [54]. Novel strategies to administer adjunctive treatment as part of host-directed therapies have been advocated to combat the shortcomings of conventional TB therapy, improving treatment success and reducing disease relapse [55, 58].

There is a need to extend the application of non-invasive hypoxia imaging for patient diagnosis, treatment and monitoring of treatment response in the realm beyond oncology. The next phase of studies evaluating the application of hypoxia-specific radiopharmaceuticals beyond oncology is necessary where the role and predictive value of hypoxia imaging in these instances is investigated [3].

Conclusion

Pre-clinical work exploring various ^{68}Ga-labelled hypoxia-seeking radiopharmaceuticals has shown promising results. Development of ^{68}Ga-labelled nitroimidazoles such as ^{68}Ga–HP–DO3A–NI and ^{68}Ga–DOTA–MN2 shows superior uptake compared to the fluorinated hypoxia PET/CT agents such as ^{18}F-FMISO and 18F-FAZA. Using TRAP as a chelator in ^{68}Ga-hypoxia labelling further improves the hydrophilicity leading to rapid clearance and decreased non-essential uptake. Although most pre-clinical work and current utilisation of hypoxia PET/CT is focused on oncologic applications, one cannot ignore the potential application in other pathologies where hypoxia has a role in the development, progression and treatment of the disease. It is, therefore, necessary to further clinical research into the imaging and quantification of hypoxic conditions in various diseases utilising ^{68}Ga-labelled nitroimidazole PET/CT. Considering the role of hypoxia in TB, it is anticipated that ^{68}Ga-labelled nitroimidazole PET/CT will provide insight into the dynamic nature of hypoxia in TB which can inform the use of adjunctive host-directed therapies. An investigation into the potential use of ^{68}Ga-labelled nitroimidazole PET/CT in TB is thus recommended.

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