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EDITORIAL

What can gallium-68 PET add to receptor and molecular imaging?

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In the last decade there has been a significant increase in the development of radiolabelled peptides for diagnostic applications, especially due to simplified methods of purification. Peptides have fast clearance, rapid tissue penetration, and low antigenicity and can therefore be produced easily and inexpensively. In addition, if the diagnostic scan is positive, the peptides can be labelled with therapeutic radionuclides (yttrium-90, lutetium-177) and used for therapy [1].

Most efforts at labelling peptides have targeted somatostatin and its receptors. Somatostatin is a regulatory peptide widely distributed in the human body. Its action is mediated by membrane-bound receptors (SSTR) that are present in normal human tissues, such as thyroid, brain, gastrointestinal tract (GIT), pancreas, spleen and kidney [2]. They are also abundant in a variety of human tumours, notably neuroendocrine tumours (NET) [3] of which carcinoid tumour and phaeochromocytoma are encountered most in clinical practice. SSTR are also expressed, with variable abundance, in renal cell carcinoma, small cell lung cancer, breast cancer, prostate cancer and malignant lymphoma [4]. Somatostatin itself has a short half-life and is rapidly degraded by enzymes; therefore analogues have been developed which mimic its effects but are resistant to enzyme degradation.

There are 5 somatostatin receptor subtypes but only subtypes 2 (SSTR2) and 5 (SSTR5) and to a lesser extent receptor subtype 3 (SSTR3) have a high affinity for commercially available synthetic analogues and even these differ in their affinity for the various receptor subtypes [5].

Developments in labelled peptides

The most commonly used somatostatin analogue is indium-111-diethylenetriaminepentaacetic acid (DTPA)-octreotide (^{111}In-octreotide) that has a high affinity for SSTR2 and lower affinity for SSTR5 and SSTR3 [6]. More recent developments include the use of 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA), a universal chelator capable of forming stable complexes with radiotracers of the metal group such as ^{111}In, ^{67}Ga, ^{68}Ga, ^{64}Cu, ^{90}Y and ^{177}Lu [7]. Newer analogues such as DOTA-Tyr3 octreotide (DOTATOC) have better uptake than ^{111}In octreotide. The phenylalanine residue at position 3 is replaced by tyrosine, making the compound more hydrophilic and increasing the affinity for SSTR2, leading to higher uptake in SSTR2-positive tumours [8].

Other peptides linked to DOTA include DOTA-octreotate, which has a very high affinity for SSTR2 [5], and DOTA-lanreotide with high affinity for SSTR5. The newest addition to these compounds is DOTA-1-NaI-octreotide (DOTANOC), which has shown a high affinity for SSTR2, SSTR3 and SSTR5. These products have high radiochemical purity and show rapid renal clearance but high accumulation in tumours with a striking superiority over standard peptides [9].

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All the above-mentioned peptides are currently labelled with ^{111}In for use in SSTR imaging. However, the physical properties of ^{111}In are not ideal for imaging and small lesions may be missed even with tomographic (single photon emission computed tomography, SPECT) acquisition. In addition, imaging with ^{111}In-peptides is usually performed over a 24-h period to allow adequate uptake in tumours and washout from normal tissues. There is therefore a need for a high definition imaging that can be performed over a shorter period of time, such as that provided by positron emission tomography (PET).

Clinical experience with ^{68}Ga-peptides

The chemistry and radiopharmacy of the germanium-68/gallium-68 generator (^{68}Ge/^{68}Ga) have been investigated since the late 1970s [10, 11]. ^{68}Ga has suitable physical properties with a high positron yield reaching 89% of all disintegrations. Its half-life of 68 min matches the pharmacokinetics of many peptides and other small molecules owing to a fast blood clearance, quick diffusion and target localization [4]. The fact that it is produced from the ^{68}Ge/^{68}Ga generator with a long half-life of 270.8 days of the parent makes it available in-house for round the clock production for more than 1 year.

The recent introduction of ^{68}Ga PET imaging in clinical practice represents a landmark in the ongoing developments in functional and metabolic imaging that is not dependent on the availability of a cyclotron. The first impressive ^{68}Ga-DOTATOC PET imaging of neuroendocrine tumours was described by Hofmann et al. [12] who compared ^{111}In-octreotide scintigraphy with ^{68}Ga-DOTATOC PET in eight patients with carcinoid tumours. ^{68}Ga-DOTATOC PET identified all 40 lesions whereas ^{111}In-octreotide (even with SPECT) identified only 85%. More importantly, quantitative analysis of the lesions showed that ^{68}Ga-DOTATOC PET imaging resulted in higher tumour to non-tumour contrast with low kidney accumulation. This has been demonstrated in pre-clinical studies [4], but carries a greater impact when is shown in humans.

Another comparison between the two radiopharmaceuticals showed that ^{68}Ga-DOTATOC PET was better at demonstrating smaller lesions with low tracer uptake [13]. The pharmacokinetics of ^{68}Ga-DOTATOC was studied by Koukouraki et al. in an attempt to establish parameters affecting the standard uptake value (SUV) in patients with metastatic NET [14]. Their dynamic qualitative analysis showed increased uptake of ^{68}Ga-DOTATOC in 21 of 22 patients and in 72 of 74 lesions with a variable SUV range (0.877–28.07, mean: 8.73). They confirmed high receptor binding and internalisation, but low cellular externalisation and relatively low fractional blood volume. This is helpful in optimizing planning for ^{90}Y-DOTATOC therapy as DOTATOC uptake in NET is mainly dependent on receptor binding and fractional blood volume, and by using pharmacokinetic data analysis, blood background activity can be separated from the receptor binding. The same group used similar dynamic analysis to compare the pharmacokinetics of ^{68}Ga-DOTATOC PET and [^{18}F]-fluorodeoxyglucose (FDG) PET in patients with metastatic NET. Qualitative analysis showed uptake of ^{68}Ga-DOTATOC in all patients in 57 of 63 lesions, while ^{18}F-FDG uptake was observed in 43 of 63 lesions, and discordant findings were seen in 6 of 15 patients [15].

Further comparison between ^{68}Ga-DOTATOC PET and ^{99m}Tc-HYNIC-octreotide was performed more recently by Gabriel et al. [16] in 88 patients with known or suspected NET. The patients were placed in three categories: those with unknown primary tumour, but with clinical or biochemical suspicion of neuroendocrine malignancy (n=13 patients), those for staging of known tumour (n=36 patients) and those being followed up after therapy (n=35 patients). ^{68}Ga-DOTATOC PET had sensitivity of 97%, specificity of 92%, and overall accuracy of 96%, and showed significantly higher diagnostic efficacy compared with ^{99m}Tc-HYNIC-octreotide scintigraphy and computed tomography (CT) ($p<0.001$). The combined use of PET and CT were shown to have the highest overall accuracy.

Another clinical application of imaging with ^{68}Ga-DOTATATE PET is in the management of phaeochromocytoma. Our group assessed the viability of such imaging in malignant phaeochromocytomas in a small group of five patients who had previously undergone surgical resection of histologically proven malignant phaeochromocytomas and subsequently presented with clinical and biochemical signs of recurrence [17, 18]. All patients underwent imaging with CT, ^{123}I-metaiodobenzylguanidine (MIBG) and ^{68}Ga-DOTATATE PET. Three patients had concordant results while two patients had negative ^{123}I-MIBG scintigraphy but positive ^{68}Ga-DOTATATE PET. The SUV (max.) for the positive lesions ranged from 4.6 to 10.4, indicating good tumour to background ratio. These findings present an interesting role for ^{68}Ga-DOTATATE PET in malignant phaeochromocytomas, especially those that show no or little avidity to MIBG. In addition this may lead to further treatment options with radiolabelled somatostatin analogues such as yttrium-90 DOTATATE [19] or lutetium-177 octreotate [20] and is particularly relevant in cases of malignant phaeochromocytoma where recurrent or metastatic disease is usually not amenable to conventional treatment strategies.

Besides the DOTA analogues of somatostatin, DOTA-related analogues of several other interesting peptides have been developed though the majority of these applications remain at the pre-clinical and research level.

Pre-clinical studies with ^{68}Ga PET

It is notable that very few pre-clinical studies, mostly with unstable compounds [21, 22], preceded the introduction of ^{68}Ga into clinical practice, which meant that such studies are still progressing alongside clinical studies. Pre-clinical studies on somatostatin analogues showed that ^{68}Ga-desferrioxamine (DFO)-octreotide injected in rats bearing SSTR-positive pancreatic tumours had a selective binding to the tumour site, with a tumour to background ratio (TBR)=5 [23].

Subsequently, somatostatin receptors were evaluated in vivo with several DOTA-related-labelled somatostatin analogues, among which ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC were the most promising [24–27].

Biologic evaluation in living rats of ^{68}Ga-DOTA-labelled oligonucleotides for labelling antisense oligonucleotides targeting activated human K-ras oncogene was recently published by Roivainen et al. [28]. They evaluated the biodistribution and biokinetics of the tracer in vivo in tumour-bearing athymic rats and showed that ^{68}Ga-DOTA-oligonucleotide compounds are stable and capable of producing high-quality PET images. Antunes et al. determined somatostatin receptor affinity by in vitro receptor autoradiography in a rat xenograft tumour model. They demonstrated that third-generation gallium-DOTA-octapeptides have distinctly better pre-clinical pharmacological performances than the indium-labelled peptides [4].

^{68}Ga has also been successfully labelled to melanocortin peptides. These are involved in many physiologic functions, and their receptors are expressed in several cells like cutaneous melanocytes, keratinocytes, fibroblasts, endothelial cells, antigen-presenting cells and leukocytes. One of the tumours that can benefit from such imaging is melanoma which over-expresses melanocortin receptor. A melanocyte-stimulating hormone (MSH) analogue, [Nle4, Asp5,D-Phe7]-MSH (4–11) (NAPamide), was conjugated to DOTA and labelled with ^{68}Ga to characterize both in vitro and in vivo the mouse B16F1 melanoma model. PET studies using ^{68}Ga-DOTA-NAPamide revealed high contrast images even at 1 h after tracer administration [29]. However, receptor density in human melanomas is much lower than that in the murine tumour model and more work is needed to improve receptor affinity in man.

Bombesin receptors are over-expressed on major human tumours, in particular prostate and breast cancer, and interest in labelling a bombesin-related carcinoma model with ^{68}Ga has been tried for the pre-clinical setting and in patients. One example is a pancreatic cancer model (AR42J) that was evaluated with ^{68}Ga-DOTAPEG2-[D-Tyr6, Ala11,Thi13,Nle14] bombesin. Studies published by Schuhmacher et al. have demonstrated good uptake by the tumour with a significant tumour to background ratio, ranging from 5.5 to 11, showing its potential role in clinical practice [30]. Promising pre-clinical studies using DOTA-related analogues of several other interesting peptides, including substance P [31], neurotensin [32] and cholecystokinin (CCK) [33], have also been published and will benefit from using similar models labelled with ^{68}Ga.

Tumour hypoxia is well known to affect response to cancer therapy and can be assessed with metronidazole, which has been recently successfully labelled with ^{68}Ga. A study by Ito et al. showed clear visualisation of various tumour cells with ^{68}Ga-metronidazole using ethylenedicysteine as a chelator [34]. The over-expression of multidrug resistance (MDR1) P-glycoprotein (Pgp) is another factor in tumour response to therapy that has been assessed with ^{68}Ga. Sharma and colleagues [35] examined cell tracer transport and biodistribution using ^{68}Ga micro-PET imaging. They concluded that this modality could enable noninvasive PET monitoring of the blood-brain barrier, chemotherapeutic regimens and MDR1 gene therapy protocols in vivo.

Pre-clinical studies were not limited to oncology, and an interesting application of ^{68}Ga involves the evaluation of infection. ^{68}Ga belongs to the same metallic group as ^{67}Ga that, as ^{67}Ga-citrate, has been used for the imaging of infection due to its binding to the circulating transferrin and avidity to transferrin receptors. ^{68}Ga has the same chemical characteristics and may have the added advantage of better resolution for the detection of infection compared to ^{67}Ga. A rat model of *Staphylococcus aureus*-induced osteomyelitis was studied by Makinen et al. using ^{68}Ga small animal PET. They concluded that ^{68}Ga PET is feasible for the imaging of bone infection and although still far from being applied in clinical practice, it has a potential role in this field [36].

Despite these encouraging prospects for the use of ^{68}Ga PET, it has been highlighted in a recent editorial in the *European Journal of Nuclear Medicine and Molecular Imaging* that no commercial body has yet obtained a marketing authorization for a ^{68}Ge/^{68}Ga generator! This, due to the requirements imposed by pharmaceutical legislation, means that ^{68}Ga-labelled compounds may not become available as a standard radiopharmaceutical for widespread use for some time to come [37]. This can, and must, be solved by the simultaneous increase in the use of the generator and constructive dialogue with the industry.

Conclusion

The recent development of ^{68}Ga PET is a true landmark in molecular imaging that will allow for the use of diverse molecules and receptor analogues in clinical practice. The inherent superiority of PET imaging is a clear advantage

compared to single photon imaging, while the feasibility of using the ^{68}Ge/^{68}Ga generator, round the clock for more than a year, is extremely cost-effective negating the need for on-site cyclotron.

The clinical application of ^{68}Ga-peptides, particularly the third generation of somatostatin analogues, has been successful in a variety of tumours, particularly NET, to the extent that its clinical application has preceded its pre-clinical assessment. Nevertheless, great interest has been shown in labelling other molecules and tumour models that will improve the management of other tumours and the assessment of infection.

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