

FOCUS ON: NEURO-ENDOCRINE TUMOURS

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Nuclear medicine imaging and therapy of neuroendocrine tumours

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Abstract

Radiolabelled peptides are used for specific targeting of receptors (over-)expressed by tumour cells. Dependent on the kind of labelling and the radionuclide used, these compounds may be utilised for imaging or for therapy. A concise overview is provided on basic principles of designing and developing radiopeptides for these applications. Furthermore, clinical application of these compounds for imaging and therapy is described. Advantages of the method compared to other techniques (such as the use of radiolabelled antibodies or antibody fragments) are discussed as well as pitfalls and limitations.

Keywords: Peptide; receptor; scintigraphy; radiotherapy.

Introduction

Radiolabelled receptor binding peptides have emerged as a new class of specifically targeting radiopharmaceuticals for tumour diagnosis and therapy. The peptides are used as transport vehicles to guide the radionuclides to the tissues expressing a particular receptor. Small peptides for receptor imaging and targeted radiotherapy have some advantages over antibodies, and even antibody fragments. Due to their small size, peptides show rapid diffusion in target tissue. They clear rapidly from the blood and non-target tissues, resulting in high tumour-to-background ratios. For conventional nuclear medicine imaging, the peptides may be labelled with γ -emitters such as ^{111}In and $^{99\text{m}}\text{Tc}$. For positron emission tomography (PET), they should be labelled with positron emitters, such as ^{18}F , ^{68}Ga , ^{64}Cu . For therapy, β -emitters are used (^{90}Y , ^{177}Lu) which will destroy tumour tissue while sparing healthy tissues, depending on the penetration range of the β -particles. To date, the ^{111}In -labelled somatostatin analogue octreotide (OctreoScan[®]) is the most successful radiopeptide for tumour imaging and has been the first to be approved for diagnostic use. Labelled with the β -emitters ^{90}Y or ^{177}Lu , it has been used for peptide receptor radiotherapy (PRRT). Other receptor-targeting peptides such as cholecystokinin

(CCK) analogues, glucagon-like peptide-1 (GLP-1), bombesin, substance P, neurotensin, and RGD peptides are currently under development or undergoing clinical trials. The basic principles for radiopeptide imaging and PRRT are the same. Therefore, both techniques are discussed with emphasis upon PRRT.

Regulatory peptides and their receptors

Regulatory peptides are potent small (30–40 amino acids) messenger molecules binding to specific G-protein-coupled receptors mainly in the brain and the gastrointestinal tract. While rapidly penetrating any tissue (except for the brain, because they cannot cross the blood–brain barrier due to hydrophilicity), they are also rapidly degraded and excreted mostly via the kidneys. The central nervous system and the periphery form two independent regulatory systems that use the same messenger molecules without danger of confusing interaction^[1–3]. While degradation and secretion is necessary for regulatory peptides to play a role as flexible messenger molecules, their use as radiopharmaceuticals is massively hampered by their short half-life in blood. Therefore, most peptides have to be modified to prevent rapid enzymatic degradation^[4,5].

Application of peptides as radiopharmaceuticals

Regulatory peptides have to be stabilised for the use as radiopeptides in order to achieve high tumour-targeting while rapid (renal) secretion is necessary to keep background activity low^[6]. In addition, during the radiolabelling procedure the peptide should preserve its receptor binding affinity and biological activity (the latter is not essential for targeting, but often goes along with affinity). To overcome the enzymatic degradation of peptides, several methods of inhibiting enzymatic degradation of peptides have been developed (binding to serum proteins will result in high background-levels which should be avoided). To achieve this goal, substitution of L-amino acids by D-amino acids, replacement of amino moieties by imino groups, substitution of peptide bonds, insertion of artificial amino acids or amino acid residues with modified side chains, amidation, cyclisation, and peptidomimetics may be used^[4,7]. Apart from stabilisation, the route and rate of excretion of peptides can be modified by introduction of specific hydrophilic or lipophilic amino acid residues into the peptide-chain^[8]. Peptides can also be modified by linking them to polyethylene glycol (PEG) chains, a technique called PEGylation^[9,10], in order to achieve stable hydrophilic peptides.

Radiolabelling of peptides

The radiolabelling procedure should not affect the receptor binding affinity of the peptide while retention of the tracer within the target cell is warranted^[11]. This can be achieved by so-called residualising labels which are retained in the cell (due to lack of a metabolic pathway) even if the peptide serving as carrier is degraded after internalisation. Radiolabelling of peptides with metals such as ¹¹¹In or ¹⁷⁷Lu is performed by conjugating peptides with bifunctional chelators that complex free metal ions. The most widely used chelators are diethylenetriaminepentaacetic acid (DTPA) (Fig. 1) and 1,4,7,10-tetraazacyclo-dodecane-*N',N'',N''',N''''*,-tetraacetic acid (DOTA). While the first is commonly used for imaging due to the simplicity of the labelling procedure, the latter is used for therapy due to the higher stability of the radionuclide-chelator complex^[12,13]. DOTA can also be used for labelling with positron emitters such as ⁶⁴Cu or ⁶⁸Ga. For labelling with ^{99m}Tc, bifunctional coupling agents may be used such as MAG₃^[14-16] or HYNIC^[17,18].

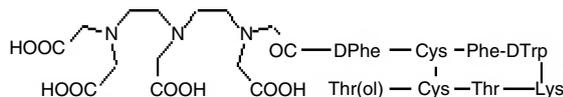


Figure 1 The chemical structure of DTPA-DPhe¹-octreotide. The chelator DTPA is conjugated to the peptide via the DPhe in position 1 of the peptide.

Somatostatins

Somatostatin is a cyclic 14 amino acid discovered in 1973. In the central nervous system, somatostatin acts as a neurotransmitter^[19]. Somatostatin receptors are expressed in most neuroendocrine tumours. Five subtypes of human somatostatin receptors (hSSTR) have been identified^[20] and natural somatostatin has a high affinity for all of them. Due to the low metabolic stability of somatostatin-14, by rational design the somatostatin analogue octreotide was developed showing enhanced stability towards enzymatic degradation^[21]. By N-terminal conjugation of octreotide to the chelator DTPA, the so-called pentreotide was developed enabling radiolabelling with ¹¹¹In^[22,23]. For PRRT, DOTA-conjugated somatostatin analogues have been developed^[24-29]. ^{99m}Tc-HYNIC-D-Phe¹-Tyr³-octreotide has been developed for imaging which shows some advantages over ¹¹¹In-labelled compounds (lower radiation exposure, higher spatial resolution)^[30,31]. For PET imaging, compounds have been developed labelled with ⁶⁸Ga, ⁶⁴Cu, and ¹⁸F^[32-39]. In comparison to conventional imaging, sensitivity is improved. Especially ⁶⁸Ga is a promising compound because it can be eluted from Ge/Ga generators even in PET centres without an on-site cyclotron.

Scintigraphic imaging of tumours

Clinical somatostatin receptor scintigraphy (SRS) with ¹¹¹In-DTPA-octreotide mainly visualises tumours expressing somatostatin receptor subtype 2 (sstr2) (and also 5 (sstr5)) as octreotide does not have a high affinity to the other somatostatin receptor subtypes^[40]. sstr 2 is expressed by a large variety of tumours, especially neuroendocrine tumours, lung cancer, breast cancer, differentiated thyroid cancers, but also meningiomas, well-differentiated astrocytomas, pituitary tumours, or malignant lymphomas and several others^[41-46]. In some gastrointestinal neuroendocrine tumours, it is considered the diagnostic gold-standard^[47-49] while in other tumours such as insulinomas or medullary thyroid carcinoma, the sensitivity is below 50%^[47,50-52]. In dedifferentiating tumours, the somatostatin receptor expression may be lost resulting in low sensitivity of diagnostic imaging while in these cases PRRT will not be of help^[52]. For imaging with positron emitters, octreotide analogues have been developed. As already stated above, these show an increased sensitivity as compared to ¹¹¹In-labelled octreotide.

Peptide receptor radionuclide therapy (PRRT)

Peptides used for PRRT need to be designed for high tumour retention. Therefore, it is crucial to use residu-

alising labels for binding of therapeutic radionuclides, usually β -emitters. The basic principles of labelling of radiopeptides have already been described. The β -emitters that are suited for therapeutic use and the most frequently used to date are ^{90}Y (β_{max} 2.3 MeV, $t_{1/2}$ 64 h), ^{186}Re (β_{max} 1.1 MeV, $t_{1/2}$ 91 h), ^{188}Re (β_{max} 2.1 MeV, $t_{1/2}$ 17 h), ^{131}I (β_{max} 0.6 MeV, $t_{1/2}$ 192 h), and ^{177}Lu (β_{max} 0.5 MeV, $t_{1/2}$ 161 h). For PRRT, ^{90}Y and ^{177}Lu have most widely been used. As high energy β radiation has a long penetration range in tissue, it is less efficient when treating smaller tumour lesions (<1–2 g) as much of the energy is deposited outside the lesion. Therefore, high energy particles such as ^{90}Y have been considered more appropriate for the treatment of larger tumours (with a heterogeneous receptor distribution) whereas low energy particles such as ^{177}Lu may be more suitable for the treatment of small lesions^[53]. Indeed, it has been shown that the combination of radionuclides with different β -energies and particle ranges may have good potential to achieve higher cure rates in tumours of differing sizes^[54]. However, clinical trials are awaited to support these findings.

Apart from β -emitters, the auger-emitter ^{111}In has also been used for PRRT; ^{111}In emits γ -rays as well as conversion and auger electrons, the latter being responsible for the therapeutic effects^[55]. Furthermore, pre-clinical data exist about the use of α -emitters (^{211}At , ^{213}Bi) for PRRT^[56,57]. α -emitters may be able to induce more damage to tissue due to the higher energy deposition in relation to the short range of about 50 μm in tissue^[58].

To date, clinical trials have been performed using mainly ^{90}Y and ^{177}Lu as emitters bound to octreotide analogues (mostly DOTA-Tyr³-octreotide (DOTATOC)^[59] and DOTA-Tyr³-Thr⁸-octreotide (DOTATATE)^[60]).

Efficacy of PRRT

PRRT is mostly performed in patients with neuroendocrine tumours of the gastrointestinal tract as well as carcinoid tumours of other localisations. The effects of PRRT—as the effects of any other anti-cancer therapy—vary dependent on the size of the tumours, the stage of disease, differentiation of the tumour cells, and other factors. Using [^{90}Y]DOTA-Toc, response rates obtained range from ~6% to ~30% for partial remissions while stable disease has been found in 52–88% of the patients^[28,59,61]. Complete remissions may be achieved in single patients. However, some studies fail to report the number of patients with progressive or stable disease prior to therapy^[28,61]. Other studies report on the use of DOTATATE for PRRT, labelled either with ^{90}Y or ^{177}Lu . Independent of the radionuclide used, the response rate is reported to be in the range of 30–40% for partial remissions and stable disease in prior progressive patients has been reported in ~40–50%^[62–64]. Randomised controlled clinical trials to find the optimal treatment

scheme for PRRT are missing so far, probably also due to the limited number of patients.

Apart from somatostatin analogues, other peptides have been used for PRRT. ^{90}Y -labelled minigastrin has been used successfully in patients with medullary thyroid carcinoma with response rates above 30%^[65,66]. The response rate dropped when ^{111}In was used instead of ^{90}Y as radionuclide in gastrin receptor-targeted therapy^[67].

Toxicity of PRRT

Haematological toxicity

Acute haematological toxicity is usually mild, no matter which of the radionuclides is used. WHO grade 3–4 toxicity may be reached in up to 15% of the patients^[61]. However, certain dosage limits need to be respected. In single patients with previous chemotherapy, myelodysplastic syndromes have been observed^[64]. Especially with ^{111}In used as radionuclide, if a limit of 100 GBq or 3 Gy bone marrow dose had been exceeded, patients developed myelodysplastic syndrome^[68].

Renal toxicity

Dose-limiting renal toxicity is probably the most important issue in toxicity of PRRT. This toxicity is attributable to the re-absorption of radiolabelled peptides in the renal tubuli via megalin^[69], leading to a relatively high radiation dose to the glomeruli that may result in an irreversible loss of kidney function. In comparison to β -emitters, the Auger emitter ^{111}In does not show considerable renal toxicity because due to the shorter range of the radiation, the glomeruli are preserved. The tubular epithelia which are damaged, on the other hand, quickly recover^[11]. Due to the better results of PRRT using ^{177}Lu or ^{90}Y , renal toxicity needs to be reduced for effective tumour treatment. Therefore, positively charged amino acids but also plasma-expanders have been used successfully to reduce kidney re-absorption of radiolabelled octreotide analogues^[70–73]. Cumulative activity of ^{90}Y applied to single patients should not exceed 7.4 GBq/m² as this will probably increase the risk of renal failure^[74].

Liver toxicity

Liver toxicity may occur in single patients with liver metastases undergoing PRRT. However, it will always remain difficult to reliably detect liver toxicity of PRRT itself because an increase in liver parameters could also be attributable to liver damage due to metastatic disease. Single patients with extensive metastases to the liver and acute liver failure, however, have been described^[61].

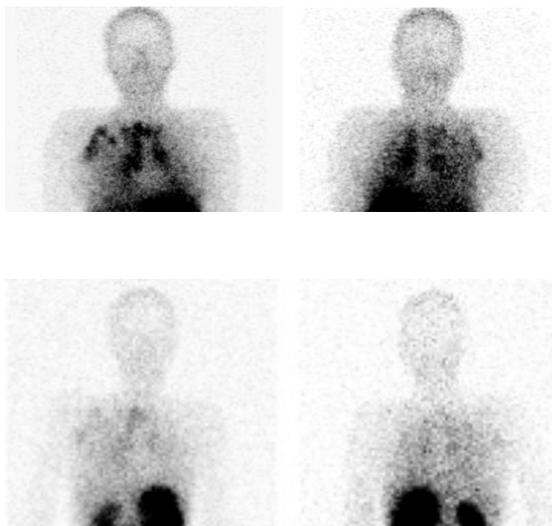


Figure 2 A patient with medullary thyroid carcinoma metastasised to the mediastinum and the right axilla. Pre-therapeutic planar scans show high uptake of $^{111}\text{In-DTPA-D-Glu}^1\text{-Minigastrin}$ into the tumours (top) while uptake is much lower after three cycles of therapy with $^{111}\text{In-DTPA-D-Glu}^1\text{-Minigastrin}$ (bottom). CT scans showed stable disease in this previously progressive patient.

Future developments

A number of new radiopeptides are currently under development. CCK_2 binding peptides have been used in imaging and therapy (Fig. 2)^[65–67,75]. Early clinical studies with bombesin analogues in patients with invasive prostate carcinoma are currently underway^[76]. ^{90}Y -labelled substance P has been used for intracavitary brachytherapy of high grade gliomas^[77] although systemic application of this compound may cause considerable side-effects^[78]. However, local application into tumour tissue does not cause these problems. In pre-clinical studies, GLP-1 analogues have been used for the detection of insulinomas and radiometal-labelled analogues have been developed^[79–81]. Recently, two studies with $^{99\text{m}}\text{Tc}$ -labelled VIP analogs in patients with high grade spindle cell sarcoma, ductal epithelial hyperplasia, and colorectal cancer suggest that this radiopeptide may be valuable for clinical application^[82,83]. Pre-clinical studies with ^{111}In -labelled DTPA- and DOTA-conjugated neurotensin analogues suggest that these may be applied in the management of patients with exocrine pancreatic cancer^[84]. Finally, RGD peptides targeting the $\alpha_v\beta_3$ integrin preferentially expressed on proliferating endothelial cells^[85] are under development. These peptides may offer a wide clinical application in quickly proliferating tumours^[86,87]. In a study with patients using an ^{18}F -labelled RGD peptide, uptake patterns were detected differing from ^{18}F FDG uptake. Therefore, this new compound will probably lead to

new insights into individual tumour biology (growth rate, neovascularisation, etc.). It may furthermore be possible to non-invasively characterise tumours for optimisation of therapy^[87].

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