Radiolabeled Peptides for Imaging and Therapy: A Radiant Future

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Running title: Novel perspectives for radiopeptides
ABSTRACT

Radiolabeled peptides (radiopeptides) are very powerful tools for diagnostic imaging and radionuclide therapy of various diseases. Since the introduction of the first radiopeptide into the clinical setting to diagnose neuroendocrine tumors (NETs) about 25 years ago, many advances have been made in the field. This short review aims to highlight novel strategies to improve the application of radiopeptides for imaging and therapy.

Key Words: radiopeptides; imaging; radionuclide therapy; developments; perspectives

SOMATOSTATIN-RECEPTOR TARGETING AS ROLE MODEL

Radiopeptides targeting the somatostatin receptors (SSTR) have brought a significant contribution to patient care. The SSTR agonist octreotide and modifications thereof ([Tyr³]octreotide (TOC), [Tyr³, Thr⁸]octreotide (TATE), and [1-Nal³]octreotide (NOC)) are widely used in routine clinical practice for diagnosis of NETs. ¹¹¹In-diethylenetriaminepentaacetic acid (DTPA)-octreotide (OctreoScan; Mallinckrodt Inc.) was the first registered radiopeptide, and it has become the gold standard for diagnosis and staging of SSTR-positive tumors using SPECT and SPECT/CT. Following the successful application of ¹¹¹In-
octreotide for imaging of NETs in the clinic, somatostatin analogs radiolabeled with β-emitting isotopes (i.e. $^{177}$Lu-DOTATATE and $^{90}$Y-DOTATOC) were introduced as treatment. Peptide receptor radionuclide therapy (PRRT) using $^{177}$Lu-DOTATATE and $^{90}$Y-DOTATOC has given impressive results thus far.

The success of radiolabeled somatostatin analogs inspired the scientific community to develop radiopeptides targeting different receptor families, including gastrin-releasing peptide (GRP), cholecystokinin-2/gastrin (CCK2/CCKB), glucagon-like peptide-1 (GLP-1), $\alpha_\nu\beta_3$-integrin, neurokinin type 1 (NK-1), melanocyte-stimulating hormone (MSH), neuropeptide Y (NPY), luteinizing hormone releasing hormone (LHRH) and chemokine 4 (CXCR4) receptors (1). Examples of radiopeptide families and their applications for tumor imaging and therapy are shown in Figure 1. Radiopeptides have established themselves as important agents for imaging, but also in PRRT. For medical imaging, peptides can be radiolabelled with radionuclides such as $^{99m}$Tc, $^{111}$In, $^{67}$Ga, or $^{123}$I for SPECT and $^{18}$F, $^{68}$Ga, $^{64}$Cu, $^{86}$Y, or $^{124}$I for PET. For therapeutic purposes, peptides can be labeled with β-emitters such as $^{177}$Lu, $^{90}$Y or $^{67}$Cu, and α-emitters such as $^{213}$Bi or $^{225}$Ac. Current applications of clinical radiopeptides for imaging and therapy have been described in detail by Ambrosini et al. (2). Approaches intending to
improve performance of radiopeptides for imaging and therapy and thereby to widen their scope of application are discussed below.

**NOVEL PERSPECTIVES FOR IMAGING**

Intensive research efforts have resulted in improved pharmacokinetics and in vivo stability of radiopeptides, as well as having boosted imaging quality by selecting radionuclides with more suitable nuclear physical properties or by combining nuclear imaging modalities with complementary modalities. Some examples of novel approaches for radionuclide therapy are shown in Figure 2.

**Change of Paradigm Using Receptor Antagonists**

An important eye-opener in the field of radiopeptides has been the recent introduction of SSTr antagonists, which showed more favorable pharmacokinetics and superior tumor visualization than agonists did, despite their poor internalization rate. In a clinical study, the SSTr antagonist $^{111}\text{In}$-DOTA-BASS resulted in higher tumor uptake and better visualization of metastatic NETs than the SSTr agonist $^{111}\text{In}$-DTPA-octreotide (3).

Subsequently, radiolabeled GRPR-targeted antagonists also entered the arena, showing a more favorable pharmacokinetic profile and fewer side effects
than agonists. GRPR-targeted antagonists have shown high potential in early clinical trials for prostate cancer imaging (4).

**Improving Pharmacokinetics and In Vivo Stability**

Many efforts to increase radiopeptide *in vivo* stability by introducing structural modifications have been made, although such modifications can cause undesired changes in pharmacokinetics and/or impaired receptor affinity. A remarkable advance in this area is the discovery of the *in vivo* enzyme inhibition concept, in which radiopeptides are administered together with an enzyme inhibitor as a safeguard against enzymatic degradation. It has recently been shown that co-injection of a neutral endopeptidase inhibitor, such as phosphoramidon (PA), can stabilize radiolabelled bombesin, minigastrin and somatostatin analogs in vivo, leading to enhanced tumor uptake and improved tumor visualization (5). We believe that this strategy will have a significant impact on patient-care, as it may enhance diagnostic sensitivity and therapeutic efficacy of radiopeptides. For that purpose, a neutral endopeptidase inhibitor already applied in patients (e.g. Thiorphan) is the preferred choice.

**68Ga Versus 18F**

Recently, imaging quality has been boosted by selecting radionuclides with more suitable nuclear physical properties. For SPECT imaging, $^{99m}$Tc is
the radioisotope of choice. However, recent developments in PET/CT and PET/MR technologies are steering the field towards positron emitters.

$^{68}$Ga-radiopeptides are useful tools for PET/CT or PET/MR imaging of diseases as $^{68}$Ga can be obtained in-house from a $^{68}$Ge/$^{68}$Ga-generator. The introduction of $^{68}$Ga-labeled DOTA-conjugated somatostatin analogs (TOC, TATE, NOC) into the clinic, enabling PET/CT imaging of NETs, is an important advance in somatostatin-based imaging. The superior performance of imaging techniques using these tracers for detection of NET lesions was described by Ambrosini et al. (2).

Recently, $^{68}$Ga-labeled exendin-4 has been introduced in the clinic for the detection of GLP-1 receptor-positive insulinomas (6). $^{68}$Ga-DOTA-exendin-4 PET/CT is preferred over $^{111}$In-DOTA-exendin-4 SPECT/CT because of the higher spatial resolution and better quantification options obtained with PET/CT cameras.

$^{18}$F-labeled peptides have some advantages over $^{68}$Ga-labeled peptides, such as a longer half-life. Moreover, physical properties of $^{18}$F are more favorable for PET imaging than those of $^{68}$Ga. Indeed, an $^{18}$F-labeled GRPR-antagonist, BAY 864367, has recently been implemented in the clinical setting (4). A drawback yet to be overcome with $^{18}$F-labeled peptides is their higher
lipophilicity, which gives rise to less favorable pharmacokinetics. An interesting approach consists of labeling peptides with Al\textsuperscript{18}F via radiometalation chemistry. Several peptides have been labeled with Al\textsuperscript{18}F, such as octreotide, E[c(RGDyK)]\textsubscript{2}, exendin-4, the bombesin analog NOTA-8-Aoc-BBN(7-14)NH\textsubscript{2}, and the GRPR-antagonist JMV4168 (7, 8). The development of a kit-based method for labeling peptides with \textsuperscript{18}F would make application of \textsuperscript{18}F-labeled peptides possible in a wider range of hospitals.

**Nuclear-Optical Multimodality Imaging**

Nuclear imaging technologies available in the clinic (SPECT/CT, PET/CT, and PET/MR) are superior to any other clinical imaging modality in terms of specificity and sensitivity. However, optical imaging offers interesting applications as well and could complement the nuclear medicine uses (9). An important development in the field of radiopeptides has been the introduction of hybrid derivatives, containing both a fluorescent and radioactive label, as these have significant implications in the field of image-guide surgery. Several hybrid radiopeptides have been developed, including agents targeting SSTr, GRPr, interleukin-11 receptor-\(\alpha\), \(\alpha\text{v}\beta\text{3}\)-integrin, matrix metalloproteinase (MMP), CXCR4, and GLP-1R (10-12). Because of the size and lipophilicity of common fluorescent dyes, the main challenge lies in the optimization of chemical structures of hybrid tracers to preserve receptor affinity and biodistribution.
patterns of the peptide-tracer and to offer maximum tissue penetration range of the dyes.

**NOVEL PERSPECTIVES FOR THERAPY**

Novel strategies might improve the outcome of PRRT. These include, but are not limited to, the improvement of pharmacokinetics and in vivo stability, combination of radionuclides, the combination of PRRT with chemotherapy, the use of alpha-emitting radionuclides, and/or the use of novel methods to predict therapy response. Recent developments to increase the therapeutic effects of PRRT have been described in detail by Bison et al. (13). Some examples of novel approaches for radionuclide therapy are shown in Figure 3.

**Improving Pharmacokinetics and In Vivo Stability**

Success of PRRT would presumably be improved by enhancing the dose delivered to the tumor and/or limiting the dose delivered to normal healthy tissues. The use of SSTr-antagonists enhanced tumor targeting and prolonged tumor retention compared to SSTr-agonists, resulting in an enhanced tumor radiation dose during PRRT. In a clinical study, the SSTr-antagonist $^{177}$Lu-DOTA-JR11 exhibited higher tumor uptake and longer intratumoral residence time than the SSTr-agonist $^{177}$Lu-DOTATATE (14). This study was performed
in only a small number of patients, so more systematic clinical studies are needed now to fully evaluate the role of $^{177}$Lu-DOTA-JR11 for therapy of NETs.

Patients with advanced prostate cancer could also benefit from PRRT, as treatment options for this patient group are limited. $^{177}$Lu-GRPr-antagonists have not been tested in clinical studies thus far, even though they have shown promising results in preclinical studies (15). Radionuclide therapy using PSMA-targeted tracers has been more widely explored, as PSMA expression has been validated in advanced prostate cancer in several reports. Extensive comparative studies showing target expression of PSMA and GRPr in advanced prostate cancer would guide the choice of therapeutic options for those patients.

**Combination Therapies**

In the search for increased therapeutic efficacy of PRRT, therapeutic radionuclides with different physical characteristics have been combined to target a wider range of tumor lesions. $^{90}$Y and $^{177}$Lu $\beta$-emitting isotopes suitable for treatment of large and small tumors have been combined in simultaneous or sequential treatment. A recent retrospective study (16) described the benefits of the combination of $^{90}$Y-DOTATOC and $^{177}$Lu-DOTATOC over treatment with either radiopeptide alone, with increased survival after the combination
treatment. However, patient selection bias cannot be excluded; therefore, prospective, randomised trials are needed before firm conclusions can be drawn.

PRRT could also be combined with other therapies to increase tumor response. Chemotherapeutics could sensitize tumor cells to PRRT by modulating DNA damage and repair mechanisms, apoptosis, cell proliferation, cell-cycle synchronization, tumor cell oxygenation, etc. However, very careful design of such combination studies is needed, as chemotherapeutics could also alter receptor expression and tumor vascularization. For instance, the DNA alkylating drug temozolomide increased tumor perfusion in a preclinical study (13). Clinical studies combining PRRT using $^{177}$Lu-DOTATATE with several chemotherapeutics have been reported. Capecitabine and temozolomide both increased response rate of $^{177}$Lu-DOTATATE in comparison with $^{177}$Lu-DOTATATE alone (17). A recent proof-of-concept study described the combination of PRRT with everolimus (18). It was shown that everolimus could be safely combined with $^{177}$Lu-octreotate to treat low-grade NETs.

Introduction of Powerful Alpha Emitters

An important development in the field of radionuclide therapy has been the introduction of $\alpha$-particle-emitting radionuclides. A first-in-human study
showed $^{213}\text{Bi}$-DOTATOC could eradicate neuroendocrine liver metastases pretreated with $^{90}\text{Y}/^{177}\text{Lu}$-DOTATOC. Only moderate acute and midterm hematological and renal toxicity was observed at effective therapeutic doses (19). Non-operable and critically located gliomas have been treated with alpha targeted therapy, by local injection of $^{213}\text{Bi}$-DOTA-substance P, providing local irradiation of the tumor. The short tissue range of $^{213}\text{Bi}$ prevents damage to adjacent brain areas. Up til now, this therapy has proven to be feasible and safe, with only mild adverse effects observed (20). Despite the very promising preliminary results obtained with $^{213}\text{Bi}$-DOTATOC and $^{213}\text{Bi}$-substance P in patients, the short half-life of $^{213}\text{Bi}$ (45.6 min) and the costs and limited availability of $^{225}\text{Ac}/^{213}\text{Bi}$ generator systems limit clinical use. The recently described accelerator-driven production of $^{225}\text{Ac}$ may allow its production in sufficient quantities to supply centers for clinical trials and open this type of treatment to a wider range of centers (21).

**Innovative Methods as Predictors of Therapeutic Response**

There is a clear need for personalization and standardization of PRRT to optimize efficacy and minimize long-term toxicity, as emphasized in a recent review of Bodei et al. (22). They recommend collecting data on several parameters to construct a patient-specific treatment plan, taking into account various patient-specific characteristics to predict probability of response and
predisposition to toxicity. Dosimetry could be used as predictor of therapy response and toxicity; a relationship between tumor-absorbed dose and response has already been demonstrated for $^{90}$Y-DOTATOC (23).

Markers of DNA-damage and repair such as phosphorylated histone variant H2AX ($\gamma$-H2AX) may predict tumor and normal-organ radiosensitivities. A recent paper described the use of a $\gamma$-H2AX-foci assay as predictor of normal-tissue toxicity after PRRT (24). Genetic components can also predict disease stage and response to therapy (25).

**CONCLUSION**

Derived from natural peptide ligands, radiopeptides can be tailored and optimized to provide personalized treatment for several types of diseases. In recent years several methods have been developed to optimize radiopeptides for imaging and therapy; these efforts and achievements may bridge the gap between the exciting promises of radiopeptides and their implementation in the clinic. Nowadays, complex multimodal ligands are being designed for theranostic applications, whereas simple radiopeptides combine all key ingredients for effective theranostic applications, including easy manufacturing, fast clearance, low immunogenicity, and efficient targeting. We believe that
recent advances as described may lead the way to a radiant future of radiopeptides in the field of theranostics.

**DISCLOSURE**

Marion de Jong and Dik Kwekkeboom own stock in Advanced Accelerator Applications (AAA). No other potential conflict of interest relevant to this article was reported.
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FIGURE 1. Examples of radiopeptide families, radionuclides and their applications for imaging of tumors. RGD = Arginylglycylaspartic acid.
FIGURE 2. (A) PET/CT (top) of insulinoma patient showing focal $^{68}$Ga-DOTA-exendin-4 uptake in distal pancreatic tail (consistent with surgically removed insulinoma) which was not shown by SPECT/CT (bottom) with $^{111}$In-DOTA-exendin-4. Images reprinted with permission of the Journal of Nuclear Medicine. (B) SPECT/CT images of mice with GRPR-expressing PC-3 tumors 4 h after injection of bombesin analog $^{111}$In-DOTA-PEG$_2$-DTyr-Gln-Trp-Ala-Val-$\beta$Ala-His-Phe-Nle-NH$_2$ without (left) or with (right) co-injection of the neutral endopeptidase inhibitor phosphoramidon (PA, 300 µg). Arrows indicate tumors.
FIGURE 3. (A) Total body images after each cycle of PRRT with 7.4 GBq $^{177}$Lu-octreotate in combination with low dose capecitabine as chemosensitizer in a patient with liver metastases of a gastrinoma. Notice the decreasing uptake
in the liver metastases, indicating and preluding a tumor response that was later confirmed with CT. (B) Course of the serum tumor markers chromogranin-A (CgA) and gastrin.
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