REVIEW ARTICLE

PEPTIDE-BASED TARGETED RADIONUCLIDE THERAPIES – SCIENCE BEHIND THE SUCCESS

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Abstract

The radionuclide therapies for solid and liquid malignancies are emerging field nowadays. The targeted radionuclide therapies have been in use since 1945. In the past 20 years, due to advancement in the nanotechnology and targeting cell receptors; radionuclide therapies have emerged as a subspecialty in nuclear medicine. Through this article, we would like to briefly describe the evolution of peptide-based radionuclide therapies, with a little emphasis on their clinical applications.

Key words: Peptide-based radiopharmaceuticals, radionuclide therapy, somatostatin-receptor-positive neuroendocrine tumour

Introduction

So far, radiotherapy techniques have proved important in treating as well as prolonging the patients' lives, depending on the type of cancer in the question. However, the success of these techniques is limited by their lack of specificity as the anticancer agents or cytotoxic technologies that do not distinguish between the cancerous regions and the normal tissues.[1] Radionuclide therapy acts the same way as the chemotherapy but targeting specific cells, by recognizing the presence or absence of specific receptors. Since the 1980s, treatment of neuroendocrine tumours has been treated using, I-131- Metaiodobenzylguanidine due to its high efficacy in treating chromaffin producing tumours (paraganglioma, pheochromocytoma and neuroblastoma).[2]

Clinical Importance of Peptide-based Radiopharmaceuticals on Cancer Therapy

Cancer therapy by the use of radionuclides involves the use of a variety of radionuclides labelled with monoclonal antibodies for specific tumours. At the basic level, this technique requires the monoclonal antibody to bind to the tumour-specific antigens to distinguish cancer cells from the normal cells and increase the therapeutic value of the radionuclides. After the first description of radio-immunotherapy by Korngold and Pressman in 1953, various radiopharmaceuticals have been developed by advanced techniques in genetic engineering and chelating methods.[3,4]

Somatisation and Somatostatin Receptors Biosynthesis

The usual biosynthesis of somatostatin happens on several sites, including the nervous system (central and peripheral nervous system), gastrointestinal tract, kidney, retina, immune cells and placenta. However, the hypothalamus forms the principle biosynthetic site for somatostatin production. The process is activated by the high cytosolic calcium concentration and membrane depolarisation in neurons and peripheral secreting cells.[5] The biosynthesis happens in two stages, where the initial stage involves the production of somatostatin peptide by ribosomal mechanisms. This phase is followed by post-translational cleavage of the peptide into smaller subsets, namely, somatostatin-14 and somatostatin-28, by the action of trypsin-like proteolytic enzymes. After synthesis, the release of SST is influenced by several chemicals, including nutrients, neurotransmitters, neuropeptides, cytokines, hormones and growth factors. Some of the examples of hormones that stimulate the release of somatostatin include the corticotrophin-release hormone, neurotensin and growth hormone-releasing hormone. On

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the other hand, some of the substances that inhibit the release of somatostatin include leptin, cytokines, opiates and gamma-aminobutyric acid (GABA).[6]

Somatostatin Receptors

There are three classifications of somatostatin receptors, including the rhodopsin-like family, the GABA-like family and the glucagon-receptor-like family that belong to the G-protein coupled receptor family. A sound understanding of the structure and function of somatostatin receptors can be achieved by exploring the G-protein coupled receptors to which they belong. G-protein coupled receptors are composed of seven transmembrane alpha helices that are linked to each other by six loops with extracellular ligand-binding domains as well as an intracellular signal transduction domain.[7] They are activated by inhibition of adenylyl cyclase, causing a decrease in the intracellular levels of cAMP and calcium channels' activity. Further subsequent events in this cascade include tyrosine phosphatases activation and the ultimate up-regulation of antimitosis. The initiation of this cascade also induces several other signal transduction pathways, including Src, Erk ½, MAP kinase, Na+ -H+ exchangers, protein kinase and p38 mitogen activation. In the end, all these pathways result in such changes as vascular contractility, ion/nutrient absorption, lowered intestinal motility, neurotransmission modulation, reduced cellular proliferation, and inhibition of exocrine/endocrine functions and among others.[8]

Subtypes of Somatostatin Receptors

At present, there are six known subtypes of somatostatin receptors, and they include ssr1, ssr2A, ssr2B, sstr3, sstr4 and sstr5. These receptors have a fair distribution across all body organs including kidney, pancreas, nervous system and the gastrointestinal tract. All the six SSRS have a structural resemblance with G-protein coupled receptors, but they have functional differences due to such variations as carboxyl terminal and amino sequences. These differences result in the differential ligand affinities as well as specificities to ligands, in addition to the differential transmission of intracellular signals after activation. In human subjects, the six receptor subtypes are also present in the above-mentioned organs in addition to several others. For example, sstr1, sstr2B, sstr4, sstr5 and sstr3 can also be found in the bronchial gland, sstr2B and sstr5 in the parotid gland and sstr1, sstr3 and sstr4 in the parathyroid gland.[9]

Somatostatin Analogues and Antitumour Effects

Both *in vivo* and *ex vivo* studies have demonstrated the antitumour activity of various somatostatin analogues. Studies on the consequences of octreotide on tumours conducted in the 1980s proved 20% partial response rate and 50% stable disease response rate.[10] In the 1990s, six studies on the effects of octreotide on advanced neuroendocrine tumours resulted in stable disease among 15%–86% of the patients. Somatostatin analogues also provide relief of such as symptoms-like diarrhoea, wheezing and flushing, which is highly associated with neuroendocrine tumours. They two analogues act in a similar way as the natural somatostatin molecules in that after binding to somatostatin receptors; they initiate cascades of events that lead to direct or indirect anti-proliferative effect. Some of the effects of these analogues that are also present after the natural secretion of somatostatin include inhibition of cell signaling, protein synthesis, hormonal secretion and increased apoptosis as well as decreased cellular proliferation.[11]

Somatostatin-Receptor-positive Neuroendocrine Tumours

One of the common characteristics of neuroendocrine tumours is that they show overexpression of somatostatin receptors. Some of these tumours include gastrinoma, insulinoma, small cell lung cancer, glucagonoma, VIPoma and phaeochromocytoma. Around 80% of all of the above named neuroendocrine tumours exhibit the expression of somatostatin receptors on their cellular membranes making them important targets in somatostatin-based radiopharmaceuticals. The gastroenteropancreatic tumours are the most common in incidence. Under the WHO classification system based on histological features gastroenteropancreatic and neuroendocrine tumours is classified into four classes, namely mixed exocrine-endocrine carcinomas, poorly-differentiated neuroendocrine carcinomas, well-differentiated neuroendocrine carcinomas and well-differentiated neuroendocrine tumours.[12]

However, most of these tumours do not produce detectable hormones, and thus they remain undiagnosed until their

advanced stages of progression when such symptoms mass effects and metastases happen. Patients with non-metastatic well-differentiated neuroendocrine carcinomas have been observed to have a survival rate of 60–100% over 5 years, but those with distant metastasis have a survival rate of 29% over a similar period. Different neuroendocrine tumours produce varying amounts of each of the five subtypes of somatostatin receptors. On average, 68, 86, 46, 93 and 57% of all neuroendocrine tumours express sstr1, sstr2, sstr3, sstr4 and sstr5 on their cells, respectively, and this shows significant importance of somatostatin receptors on their diagnoses. VIPoma is the only tumour that expresses all the sstr from type 1–5 [Table 1].[13]

The Composition of Peptide-based Radiopharmaceuticals

Peptide-based radiopharmaceuticals comprise four parts that include a radionuclide, a targeting peptide, a bifunctional chelating agent and a linker moiety.

Targeting Peptides

A targeting peptide is a delivery vehicle that takes a radionuclide to a specific antigen expressed by tumours. Most targeting peptides are regulatory peptides that can be synthesised by solid-phase peptide synthesis. These peptides can also be modified further to incorporate prosthetic groups, spacer moieties and chelating agents. Furthermore, these peptides are also chemically stable meaning that they can undergo radiolabeling processes without recording significant changes. Their small sizes also allow for a rapid clearance to facilitate diffusion of radiopharmaceuticals to the tumour as well as its excretion to avoid residual effects. However, most of these peptides are subject to biodegradation by various peptidases present in different biological systems. As a result, bio-engineered monoclonal antibodies form the other preferred option.[14]

Bifunctional Chelating Agents

Bifunctional chelating agents provide a conjugation bridge between the targeting peptide and the radionuclide to prevent direct contact between the two components that would minimize the binding affinity of the biopharmaceutical for the targeted receptor. The choice of a bifunctional chelating agent depends on the oxidation state of the metallic radionuclide in the question, but in the end, it should allow for coupling with the peptide as well as a stable compound of the radiometal. Furthermore, the resultant radionuclide-chelate complex should be biologically inert to avoid competition of binding between the target receptor and the endogenous plasma proteins.[15]

Three chelating systems may be utilised by bifunctional chelating agents. One of them is the acyclic chelating system, which forms a complex between a radiometal and open-chain polyaminopolycarboxylates under mild conditions for temperature-sensitive biomolecules and fast metal-binding kinetics for short-lived radiometals. Two of the most common example of acyclic chelators for radiopharmaceutical applications include diethylenetriamine pentaacetic acid (DTPA) and ethylenediamine tetraacetic acid.[16]

Another chelating system utilised by the bifunctional chelating agent is the macroacyclic system. The product of the macroacyclic chelating systems is kinetically more inert and thermodynamically more stable than acyclic chelating systems. Some of the common macroacyclic chelating agents include triaza, cyclen-type and cyclam-type tetraaza chelators. For example, DOTA (cyclen-type 1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetic acid), which is ubiquitous for +3-charged radionuclides.[16]

Bioconjugation

The conjugation of bifunctional chelating agents with a peptide can also be achieved by conjugating to a peptide sequence through the side chain functionalities of the amino acids or the primary N-terminal amine. Some of the conjugation strategies use to produce bioconjugation, in this case, include utilising azides, maleimides, N-hydroxysuccinimide esters and isothiocyanates. Of all these examples, the N-hydroxysuccinimide esters are the most commonly used especially after their activation to form a primary amine of the amine or lysine group at the peptide sequence's N-terminus.[17]

Linkers

A linker is a radiopharmaceutical component that has two orthogonal sites of conjugation for connection between the targeting peptide and the radionuclide-chelate compound. It serves as a spacer between the radionuclide and the targeting peptide to prevent binding interference

Table 1: Percentage of cells that express SSTR in GI neuroendorine tumours[13]

Tumour type	Percentage of cell that express sstr				
	sstr 1	sstr 2	sstr 3	sstr 4	sstr 5
Insulinoma	33	100	33	100	67
Gastrinoma	33	50	17	83	50
Glucagonoma	67	100	67	67	67

between the radionuclide and the targeting peptide and sustains a high binding affinity for the targeted receptor and the peptide. If the linker is electrically charged, hydrophilic or lipophilic, it modifies the pharmacokinetics of the conjugate to influence its clearance from the body, excretion or uptake by the tumours. In addition, a linker provides an avenue for the creation of a bifunctional radioconjugate by providing additional functionality. One of the commonly used linkers is beta-alanine.[17]

Clinically Available Radiopharmaceuticals Yttrium-90 (Y-90)

Yttrium-90 is a short-lived beta-emitting radionuclide that can be milked and purified from its parent radioisotope and strontium-90. Its β-particles have the maximum energy of 2.27 MeV and tissue penetration of 12 mm. with half-life of 64 h. At present Y-90, a radioactive isotope used in drugs for the treatment of various cancers, including lymphoma, leukaemia, ovarian, colorectal, pancreatic and bone cancers.[18]

Lutetium 177 (Lu-177)

Lu-177 produced through three approaches, namely, charged particle acceleration and neutron irradiation in a nuclear reactor and a cyclotron. The radioactive decay of Lu-177 results in the emission of two primary radio particles, namely, gamma radiation (γ-photon) and beta-particles. Of the beta particles emitted during radioactive decay of Lu-177, 498 keV accounts for 79.3% and 380 keV accounts for 9.1% while 176 keV accounts for 12.2% of all beta-emissions by abundance.[19]

Gallium-68

The physical half-life of gallium-68 is 67.71 min, and it makes it compatible with the pharmacokinetics characteristics of such low molecular weight radiopharmaceuticals as peptides, antibody fragments, oligonucleotides and aptamers. 89% of gallium-68 decay happens through positron emission, while the rest 11% happens by electron capture. On average, the mean positron energy emitted by gallium-68 in every disintegration is 740 KeV. 68Ge/68Ga generator is almost an ideal generator strategy due to the long half-life (270.95 days) of Ge-68 (parent radionuclide) and the short half-life (67.71 min) of the resultant radionuclide (Ga-68) (Roesch and Riss, 2010). The Ga-69(p,2n) Ge-68 reaction in proton accelerator is the preferred production route of Ge-68, and it requires the use of ion exchange chromatography as a perfect separation system that prevents the break-through between the parent radionuclide and the soluble daughter.[20]

Radiolabelled Somatostatin Analogues

Octreotide, lanreotide and vaprotide

Octreotide has a long cyclic structure, and its chemical properties are similar to those of somatostatin making it a somatostatin agonist. Octreotide has been clinically tried in tumour treatments where it has been shown to over resolutions for such conditions as thyrotrophinomas and carcinoid syndrome. One of the radionuclides conjugated to octreotide to produce a radiolabelled somatostatin analogue for radionuclide targeted therapies is indium-111. This conjugation provides 111In-DTPA 0, octreotide, which emits Auger electrons and gamma-rays after administration in patients with metastatic tumours. Lanreotide is a somatostatin analogue with similar properties as octreotide as well as long-acting pharmacological properties. Lanreotide can be treated with indium-111 and yttrium-90 to produce two radiolabelled somatostatin analogues, namely, 111In-DOTA-lanreotide and 90Y-DOTA-lanreotide, respectively. 111In-DOTA-lanreotide derives its tumour therapeutic effects from the natural antitumour effects of lanreotide as well as the emission of Auger electrons and gamma-rays from

after radioactive decay of indium-111. On the other hand, 90Y-DOTA-lanreotide acts in the same way, but its yttrium-90 emits beta particles.[21]

Radiolabelled somatostatin analogues and radio imaging

Since neuroendocrine tumours express more somatostatin receptors than the normal tissue, somatostatin analogues have been seen as important molecules in for localizing the tumour by the radio imaging protocol. Among all the five major subtypes of somatostatin receptors, sstr2 is the most overexpressed receptor in neuroendocrine tumours. Somatostatin imaging is mainly applied for four major reasons, including the determination of the sstr status about disease treatment, accurately stage the disease, follow-up on the disease and restage the disease. Octreotide was the first somatostatin analogue to be used in single photon emission tomography somatostatin receptor analogue imaging in the late 1980s.[22] Octreotide exhibits a high affinity for sstr5 and sstr2, but a lesser affinity for sstr3 is making it a modern fit for imaging neuroendocrine tumours. Due to this affinity for sstr2 and sstr5, a radiolabelled octreotide complex with indium-111 was developed. This complex has 68-h long half-life and when used for imaging an allowance of 24-48 h is necessary to ensure hepatobiliary and renal clearance to reduce the background activity.[23]

Conclusion

On the new horizon of personalised medicine, radioactive material can be coupled with tumour-specific targeting agents such that imaging biomarkers of the disease can be combined with radionuclide therapy. The other members of health-care team need to be educated about the benefits and risks for such procedures. We need to adopt a practical approach for manufacturing and delivering radiopharmaceuticals, assessing patient eligibility, ensuring post-therapy follow-up and addressing cost issues, which will be essential for success. The question that is likely to arise now is not, can we do it but rather, where does it fit into our treatment strategy and how do we best implement it?

Conflict of Interest

The authors declare that they have no conflict of interest.

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