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ARTICLE

Targeting prostate cancer with radiolabelled bombesins

Theodosia Maina*, Berthold Nock* and Stephen Mather†

**Institute of Radioisotopes – Radiodiagnostic Products, NCSR 'Demokritos', Athens, Greece; †Centre for Cancer Imaging, Barts and the London Queen Mary School of Medicine and Dentistry, London, UK*

Corresponding address: Prof Stephen J Mather, Department of Nuclear Medicine, St Bartholomew's Hospital, London EC1A 7BE, UK. E-mail: Stephen.Mather@cancer.org.uk

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Abstract

The fact that a number of common human tumours, including those of breast and prostate, express increased levels of the gastrin-releasing peptide receptor (GRP-R) means that this receptor is a potential target for peptide receptor mediated scintigraphy and targeted radionuclide therapy. Although clinical application is yet in its infancy, there is a considerable literature on preclinical studies aimed at developing suitable radioligands for potential clinical application. This brief review provides an overview of this research and also describes some of the limited clinical studies that have been published.

Keywords: *Bombesin; gastrin-releasing peptide; imaging; prostate; cancer.*

Introduction

Bombesin (BB) is a linear tetradecapeptide with the sequence Glu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 first isolated from the skin of the European frog *Bombina bombina*[1]. Bombesin and the corresponding mammalian peptides gastrin-releasing peptide (GRP) and neuromedin B (NMB) exert a variety of physiological actions in the nervous system and the gut. These actions are elicited after binding to G-protein coupled receptors which are situated at the outer membrane of target cells and comprise three members in mammals, the GRP-receptor (GRP-R) with high affinity for the GRP, the NMB-receptor (NMB-R) with high affinity for NMB and the orphan BB_3-receptor (BB_3-R), for which an endogenous ligand has not yet been identified. Amphibian bombesin displays a high affinity for all the above receptor subtypes[2,3]. The role of bombesin-like peptides, and especially of GRP and its interaction with the GRP-R, in promoting tumour growth in human cancer cells both in culture and in nude mice xenografts has been established by numerous studies[4–6]. Most interestingly, the expression of GRP-Rs has been documented in several frequently occurring human cancers, such as in prostate and breast carcinomas[7–11].

As a result, bombesin antagonists and anti-GRP/GRP-R antibodies have been used for treatment of GRP-R-expressing malignancies[12,13]. In an alternative strategy, bombesin-like peptides have been employed as molecular carriers of cytotoxic drugs or diagnostic/therapeutic radionuclides to GRP-R-expressing tumours for diagnosis and therapy[14–17]. For this purpose, agonists are usually preferred, due to their tendency to rapidly internalise after binding to their receptor resulting in increased target accumulation[17–20]. A brief discussion will follow on the attempts so far undertaken to obtain clinically useful GRP-R-targeting radiopeptides, comprising radiohalogenated, technetium, bi- and trivalent radiometal carrying groups of compounds.

Development and preclinical studies on radiolabelled bombesins

The prime aim of any radiopharmaceutical development programme is to produce a metabolically robust radiotracer having a well-defined structure that shows

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the maximal uptake in target tissues (i.e. tumour in this instance) and minimal uptake in normal, non-target tissues including those involved in metabolism and excretion of the tracer. In addition to selection of a receptor binding sequence and a suitable radionuclide, a labelling strategy must be developed which results in a hydrophilic conjugate that is excreted through the renal system and not the hepatobiliary/gastrointestinal tract. First attempts to radioiodinate bombesin for clinical oncology SPECT (single photon emission computed tomography) imaging applications were reported more than a decade ago[21,22]. The first *in vivo* study in an ovarian carcinoma nude mouse model emphasised the need for using prosthetic groups for obtaining *in vivo* stable radioiodinated bombesins[23]. For PET (positron emission tomography) studies, ^{18}F with its half-life of 109.7 min and low ß$^{+}$-energy (0.64 MeV) represents the ideal radionuclide[24]. The first ^{18}F-bombesins were recently evaluated in mice bearing human GRP-R-expressing xenografts[25]. In view of the expanding use of PET cameras in combination with new elegant radiofluorination techniques, it is to be expected that new, improved ^{18}F-based bombesins for oncological PET applications will soon appear. In view of the availability of iodinated analogues, it may also be feasible to use I-124 for PET imaging.

A special category of bombesin-based radiopeptides are those labelled with ^{99m}Tc due to the preeminence of ^{99m}Tc in diagnostic nuclear medicine[18–20,26]. Recent advances in SPECT technology, such as dynamic SPECT and/or fused SPECT/CT imaging, are expected to rapidly boost the evolution of ^{99m}Tc-based bombesin radiotracers toward clinically useful compounds. By attaching the strong N_2S_2 (DADT) ^{99m}Tc-binding centre to [Lys^3]BB a $^{99m}TcO^{3+}$-chelated analogue was obtained but this showed high radioactivity accumulation in the abdomen[27]. By coupling DADT to [DTPA^1,Lys^3,Tyr^4]BB a much more hydrophilic radiotracer was obtained due to the presence of four pendant DTPA^1-carboxylate groups. This analogue showed good GRP-R-targeting in mice and excretion via the renal pathway[28,29]. Neutral $^{99m}Tc^{V}O^{3+}$-complexes are also obtained by coupling tripeptide N_3S-donors to BB or BB(7-14), either directly or via a spacer[30–34]. For example, ^{99m}Tc-RP527, a BB(7-14) analogue linked at the N-terminus via a glycine-5-aminovaleric acid spacer to the tri-peptide chelator, dimethylglycyl-L-ser-L-cys, reached a 2%ID/g at 1 h pi in PC-3 xenografts in SCID (severe combined immunodeficiency) mice[30,31].

Bombesins incorporating hydrophilic [*trans*-$^{99m}Tc^{V}O_2]^{+}$-cores have also been investigated. Thus, coupling of air-stable hydrophilic phosphine containing P_2S_2-chelators to BB(7-14) yielded radiotracers of high receptor affinity and good GRP-R-targeting in mice[35,36]. Alternatively, acyclic tetraamines have been coupled to both BB and BB(7-14) to afford high specific activity radiotracers which localised in high percentage in human PC-3 xenografts in nude mice (up to 11%ID/g at 1 h pi)[37]. In view of the toxicity risks inherent in the intravenous injection of pharmacologically active peptides in humans, an acyclic tetraamine was also coupled to the potent antagonist [(D)Phe^6,Leu-NHEt^{13},*des*-Met^{14}]BB(6-14). The resulting radiopeptide exhibited impressively high uptake in PC-3 xenografts (16%ID/g at 1 h pi) in nude mice[38,39].

A versatile route for labelling bombesin via the $[^{99m}Tc^{I}$-*fac*-$(CO)_3(H_2O)_2]^{+}$-synthon[40] involved coupling of N$^{\alpha}$-histidinyl-acetic or 2-picolylamine-N,N-diacetic acid to BB(7-14)[41,42]. But the resulting radiopeptides failed to accumulate effectively in PC-3 xenografts in nude mice (<0.6%ID/g at 1.5 h pi). However, convincingly high uptake in PC-3 xenografts (up to 3.7%ID/g at 1 h pi) was exhibited by $[^{99m}Tc^{I}$-*fac*-$(CO)_3$(X)-Dpr-$(Ser)_3$]BB(7-14) analogues (X = H_2O or $P(CH_2OH)_3$)[43,44].

These studies have resulted in the availability of clinically useful ^{99m}Tc-labelled GRP-R-specific radiotracers as described below. Furthermore, owing to the similarities of technetium and rhenium chemistries these studies also assist the development of matched-pair ^{186}Re/^{188}Re-bombesin radiotherapeutic agents[45].

Another often used diagnostic radionuclide is the cyclotron produced ^{111}In. In targeted radionuclide therapy, peptide analogues labelled with ^{111}In are often used as surrogates to determine the biodistribution and dosimetry of therapeutic radiopharmaceuticals radiolabelled with bi- and trivalent metals[46,47]. For this purpose, DTPA (DTPA = diethylenetriaminepenteacetic acid) or macrocyclic derivatives like the universal chelator DOTA (DOTA = 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid) are coupled at the N-terminus of peptides[47]. Such DTPA- and DOTA-derivative bombesins afford very hydrophilic ^{111}In-radiotracers with good *in vivo* GRP-R-targeting in animal models[48–52]. DOTA-derivative bombesins have been used for stable binding of therapeutic radiometals, such as $^{90}Y^{+3}$ and the radiolanthanides $^{177}Lu^{+3}$, $^{149}Pm^{+3}$ and $^{53}Sm^{+3}$, to achieve GRP-R-targeted radiotherapy of tumours[52–55].

Bi- and trivalent radiometals, like $^{64}Cu^{+2}$ and $^{68}Ga^{+3}$, have also been used for labelling bombesins for PET applications[56–62].

Clinical studies with radiolabelled bombesin analogues

Detailed published accounts of clinical imaging studies of labelled GRP analogues describe the use of two such tracers. The first of these was ^{99m}Tc-RP527, developed by the Canadian Biotech company Resolution Pharmaceuticals Inc. Two papers by Van de Wiele have been published describing small-scale studies with this agent[63,64]. A study in six normal volunteers[63] reported

the biodistribution by planar gamma camera imaging up to 48 h pi of 555 MBq of HPLC purified material. The tracer showed rapid blood clearance and a mixed route of hepatobiliary and renal excretion with diffuse uptake and retention only in the normal breast in women and the testes in man. No side effects were observed and a low effective radiation dose of 0.01 mSv/MBq was calculated. A second study[64] was performed in 10 patients, four with prostate cancer and six with breast cancer. Planar and SPECT images were acquired one and 5–6 h pi of 555 MBq of HPLC purified material. ^{99m}Tc-RP527 showed selective uptake in one of the four prostate cancer patients all of which had androgen-resistant disease with bone metastases. In this patient about half of the bone lesions were imaged. Four of the six patients with breast cancer showed tumour uptake all involving lymph nodes and bone metastases. Tumour identification was clearer on the later images due to a decrease in background activity over this time. Normal uptake was seen in the organs of hepatobiliary and renal excretion as well as in the pancreas and normal breast tissue in some subjects. The conclusions drawn from these studies were that the agent was safe for administration and showed promise for imaging of GRP-expressing tumours but that a more extensive study was required to assess the clinical potential of the tracer and to correlate the *in vivo* uptake of the tracer with the presence of the receptor as measured on resected tissue samples after imaging. Shortly after these studies were completed, Resolution Pharmaceuticals and the rights to RP527 were acquired by Bracco Diagnostics Inc. Bracco have recently begun a phase I study of an Lu-177 labelled bombesin analogue based on RP527[54] (^{177}Lu-DOTA-[4-aminobenzoyl]–BB(6-14)) (K. Linder pers. comm.).

The second analogue to be studied in some detail was developed as a result of an academic collaboration between NCSR 'Demokritos' in Athens and Universita La Sapienza in Rome. The peptide which has the sequence cys-(6-amino-n-hexanoic acid)–BB(2-14) can be efficiently radiolabelled with ^{99m}Tc[65] although the nature of the chemical complex so formed has not been characterised. This group have studied the use of their compound in breast, prostate and colorectal cancer. Dynamic and static planar imaging was performed in five patients with primary breast cancer up to 3 h pi of the radiopeptide labelled with 185–300 MBq of ^{99m}Tc. All five primary lesions were detected as well as axillary involvement in two patients. No uptake was seen in a fibroadenocarcinoma in a contralateral breast in one patient. Diffuse uptake was observed in the ducts of normal breasts. Using a prototypic miniaturised gamma camera, the authors were able to use their tracer to guide biopsy of imaged lesions and showed a good correlation between tracer uptake and malignancy[66]. Ten patients with primary prostate tumours, two with benign adenoma and eight with cancer, were studied by dynamic, planar and SPECT imaging[67]. Neither patient with adenoma showed uptake of the tracer but all eight patients with cancer showed uptake in the prostate fossa even two in which TRUS (transrectal ultrasound) was negative. Three of these patients also showed uptake in the obturator nodes. Thirteen patients, seven with known rectal cancer and six scheduled for endoscopic removal of polyps were also studied by dynamic, and early planar and SPECT imaging up to 1 h pi[68]. Cancers were detected in 11 patients with malignant disease including one with a polyp showing severe dysplasia. Five patients showed nodal uptake of the tracer indicative of metastases which was confirmed at surgery. Scans were falsely positive in two patients with benign disease but true negative in four. In several instances Scopinaro and colleagues report a very rapid uptake of lesions by their tracer which is detected by their dynamic acquisitions within the first minutes after administration. In a recent publication[69] they summarise the kinetics of uptake in 26 patients of various pathologies showing that the time to 80% of maximal uptake was, in most cases, only a few minutes. Little information is supplied, however, on the pharmacokinetics and pattern of biodistribution and excretion in non-tumour tissues, but it appears that again a mixed pattern of excretion is present with clearance via both hepatobiliary and renal routes.

Although not described in any great detail, a number of PET imaging studies have also been performed using ^{68}Ga-DOTA bombesin analogues. For example Maecke *et al.* studied 11 patients with prostate cancer[58] and imaged the primary tumour in all patients within 30 min of injection. In three lymph node metastases were also found. Clearance of the tracer was entirely via the kidneys. In some patients a mild reversible systolic blood pressure reduction was observed in the first 2 min after administration and, in some, a significant uptake in pancreatic region was observed. Pancreatic uptake was also observed by Fröberg *et al.* after injection of ^{111}In or ^{99m}Tc labelled bombesin analogues[70]. Brief reports of imaging with ^{68}Ga-DOTA bombesin analogues have also appeared in abstract form[71,72].

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