

Research Highlights

Highlights from the latest articles on bombesin-based radiopeptides for prostate cancer diagnostics



Correct staging of prostate cancer is an unmet clinical need. Anatomical imaging methods (e.g., CT and MRI) tend to understage prostate cancer due to poor sensitivity for soft-tissue metastases. A significant number of patients with extraprostatic disease nowadays undergo noncurative surgery owing to false-negative diagnosis [1]. The utility of ^{18}F -fluorodeoxyglucose with PET or PET/CT for prostate cancer is limited because of low glucose utilization, leading to insufficient ^{18}F -fluorodeoxyglucose accumulation [2]. An alternative approach to the visualization of prostate cancer is radionuclide targeting of receptors that

are overexpressed in this malignancy, for example, the prostate-specific membrane antigen or gastrin-releasing peptide receptor (GRPR). GRPR is expressed at high density on the cell membranes of both primary and metastatic prostate cancer cells, whereas normal prostate tissue and, in most cases, benign prostate hyperplasia are predominantly GRPR-negative [3]. The most promising class of imaging agents for the visualization of GRPR are analogs of amphibian peptide bombesin (BBN) [4]. Several research papers dealing with different aspects of development of BBN-based imaging agents have been published in recent months.

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Spacer as a biodistribution modifier for $^{99\text{m}}\text{Tc}$ -labeled bombesin

Evaluation of: Liolios CC, Fragogeorgi EA, Zikos C *et al.* Structural modifications of $^{99\text{m}}\text{Tc}$ -labelled bombesin-like peptides for optimizing pharmacokinetics in prostate tumor targeting. *Int. J. Pharm.* doi:10.1016/j.ijpharm.2012.02.049 (2012) (Epub ahead of print).

The use of $^{99\text{m}}\text{Tc}$ -labeled bombesin analogs is attractive as it permits using more available SPECT and SPECT/CT imaging for the diagnosis of prostate cancer. The general structure of GRPR-targeting agents contains a recognition moiety based on full-length BBN peptide (BBN[2–14]) or on its truncated part (BBN[7–14]), a spacer, and a chelator for the attachment of radioisotope. A spacer between the targeting part and chelator is necessary, as direct conjugation of a chelator to BBN causes an undesirable reduction of affinity. Despite low involvement of a spacer in GRPR binding and labeling, it can influence important properties of imaging agents such as affinity to GRPR,

biodistribution profile, blood clearance rate and elimination pathway.

An influence of a spacer on BBN(2–14) labeled with $^{99\text{m}}\text{Tc}$ using a glycine–glycine–cysteine peptide-based chelator was studied. Earlier, BBN(2–14) with a spacer containing three positively charged amino acids ornithines (BBN-O) showed superior tumor uptake and faster overall clearance compared with its analogs without a spacer or with a negatively charged one. In the presented study, this conjugate was compared with a homologous variant containing a tripeptide spacer constituted from more polar arginine (BBN-A). Both compounds were labeled with $^{99\text{m}}\text{Tc}$ in a high yield, and demonstrated a high binding capacity to GRPR *in vitro* (IC_{50} in low nanomolar range). Unexpectedly, in biodistribution experiments a more polar arginine-based spacer shifted the excretion pathway from predominantly renal for the ornithine spacer to a hepatobiliary one. Clearance of $^{99\text{m}}\text{Tc}$ -BBN-A from the blood and other tissues was more rapid than clearance of $^{99\text{m}}\text{Tc}$ -BBN-O. However, release of radioactivity from tumors was much faster for $^{99\text{m}}\text{Tc}$ -BBN-A. As a result,



the tumor-to-blood ratio for ^{99m}Tc -BBN-A was more than sevenfold lower than for the ornithine-containing counterpart. This study reflects the complexity of

design of BBN-based tracers, where a variety of factors influence tumor accumulation and imaging properties (contrast) of the targeting peptide.

Interplay of size and composition in targeting of GRPR using ^{99m}Tc -labeled BBN

Evaluation of: Okarvi SM, Jammaz IA. Preparation and evaluation of bombesin peptide derivatives as potential tumor imaging agents: effects of structure and composition of amino acid sequence on *in vitro* and *in vivo* characteristics. *Nucl. Med. Biol.* doi:10.1016/j.nucmedbio.2012.01.002 (2012) (Epub ahead of print).

In this study, six different truncated variants of BBN were compared with full-length BBN. All synthesized peptides were labeled with technetium- 99m using a mercaptoacetyl-tri-glycine peptide-based chelator. In order to make the peptides more hydrophilic, mono- or di-Asp spacers were introduced. In truncated variants, several amino acids

were modified with the aim of increasing metabolic stability in blood. All studied peptides were successfully labeled with technetium and demonstrated high stability of label to cysteine challenge and to metabolic degradation. Although all tested peptides had low nanomolar affinity to GRPR on living cells, all truncated versions had worse affinity than the full-length one. Use of a di-Asp spacer improved the affinity of truncated variants in comparison with a mono-Asp spacer, which was in agreement with the literature data. Lipophilicity of all truncated variants was higher than that for full-length peptide and $\log P$ varied from -1.15 to $+0.78$. Surprisingly, differences in $\log P$ were not reflected in biodistribution profiles. *In vivo* studies did not show differences in radioactivity uptake in liver or in radioactivity accumulation in intestine content between variants with

different overall lipophilicity. However, an introduction of lipophilic Phe¹² in a binding moiety dramatically increased liver uptake and hepatobiliary excretion. At the same time, the introduction of hydrophilic and a negatively charged mono- or di-Asp spacer only shifted excretion to kidney minimally. Even a small decrease in affinity translated to lower tumor uptake and faster radioactivity release from tumors. Tumor uptake of all studied peptides was specific, but uptake values were low or moderate. The best GRPR-targeting peptide in this study was full-length BBN, which demonstrated the highest hydrophilicity, the best affinity to receptors, the lowest liver uptake and level of radioactivity accumulation in intestine content, as well as the highest and most stable radioactivity uptake in tumors and the best tumor-to-blood ratios.

Influence of a linker between peptide and chelator on the targeting properties of ^{64}Cu -labeled BBN analogs

Evaluation of: Nanda PK, Pandey U, Bottenus BN *et al.* Bombesin analogues for gastrin-releasing peptide receptor imaging. *Nucl. Med. Biol.* 39(4), 461–471 (2012).

Until recently, there was a paradigm that suggested that agonistic peptides are preferable as targeting agents for imaging. However, there is growing evidence that antagonists have an appreciable imaging potential. For BBN, this is of particular interest, since the potent physiological action of this peptide restricts the injected dose of agonistic tracers. ^{64}Cu is a long-lived ($T_{1/2} = 12.7$ h) positron-emitting

radionuclide that permits the use of a central dispensary for preparation and distribution of tracers for PET. This paper evaluated three variants of ^{64}Cu -labeled antagonistic BBN analog (NO2A-X-D-Phe⁶-BBN(6–13)NH₂, where NO2A is 1,4,7-triazacyclononane-1,4-diacetic acid, and X is a spacer (6-aminohexanoic acid, 8-aminooctanoic acid or 9-amino-nonanoic acid). In this study, the influence of a spacer on affinity was minimal and all labeled variants demonstrated IC₅₀ values of 5–6 nM (difference was probably within accuracy of the method). On the other hand, there was clear influence

of a spacer on cellular processing of tracers by PC-3 prostate cancer cells *in vitro*. Although the internalization was much less pronounced in comparison with agonists, fourfold higher radioactivity was internalized in the case of the longest spacer in comparison with shortest one. The externalization was slowest for the longest linker as well. There was clear influence of spacers on biodistribution. Particularly, blood clearance rate decreased with the increase of the length of an aliphatic spacer, while both liver uptake and extent of hepatobiliary excretion increased. At the same time, there



was no pronounced influence of spacer on tumor uptake (difference was within error of measurement). This resulted in appreciably (up to twofold) better tumor-to-organ ratios for the conjugate with

6-aminohexanoic acid spacer. This study demonstrates that a spacer between chelator and peptide can be used for modification of biodistribution of a targeting agent and optimizing of its imaging properties.

The use of BBN-RGD heterodimer may improve sensitivity of tumor imaging

Evaluation of: Jackson AB, Nanda PK, Rold TL *et al.* ^{64}Cu -NO2A-RGD-Glu-6-Ahx-BBN(7–14) NH_2 : a heterodimeric targeting vector for positron emission tomography imaging of prostate cancer. *Nucl. Med. Biol.* 39(3), 377–387 (2012).

Heterogeneity of a target expression remains a challenge when radionuclide imaging is used for tumor staging. Metastases with low expression might be missed, leading to false-negative findings and understaging. To overcome this issue, multiple scans with tracers binding to different molecular targets might be required, which would create appreciable economic and logistical drawbacks. An interesting

approach to handle this dilemma is the development of heterodimeric peptides capable of binding to two different unrelated molecular targets in tumors. One example is development of conjugates containing both BBN and RGD (Arg-Gly-Asp) peptide. The RGD peptide binds to the $\alpha_v\beta_3$ integrin, which is overexpressed in the neovasculature. Several variants of radiolabeled RGD-BBN peptides were evaluated earlier [5–8]. However, a substantial influence of the labeling approach on targeting properties requires further optimization. Jackson and co-workers evaluated a ^{64}Cu -labeled, NO2A-conjugated RGD-Glu-6-Ahx-BBN(7–14) NH_2 variant. Unlike a previously evaluated homologous heterodimer-labeled via Bn-NOTA [6], this variant did not contain

a lipophilic benzyl moiety in the chelator and is neutral. Affinity of the new conjugate to PC-3 prostate cancer cells was in a single-digit nanomolar range. In mice bearing a PC-3 xenograft, ^{64}Cu -NO2A-RGD-Glu-6-Ahx-BBN(7–14) NH_2 have demonstrated a clear capacity for tumor imaging. In comparison with a monospecific ^{64}Cu -NO2A-6-Ahx-BBN(7–14) NH_2 , ^{64}Cu -NO2A-RGD-Glu-6-Ahx-BBN(7–14) NH_2 demonstrated lower uptake in liver and intestines. Tumor uptake of ^{64}Cu -NO2A-RGD-Glu-6-Ahx-BBN(7–14) NH_2 at 24 h was twofold higher than uptake of its monospecific counterpart targeting BBN only. This study provides additional rationale for the development of bispecific imaging peptides for tumor staging.

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