SYNTHESIS AND RADIOPHARMACOLOGICAL EVALUATION OF 18F-LABELED BOMBESIN ANALOG [18F]BX-00374436 FOR IMAGING OF GRP RECEPTOR-EXPRESSION PROSTATE CANCER

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Objectives: Over the last two decades, radiolabeled peptides have been widely used as promising radiotracers for tumor targeting. In recent years, bombesin and bombesin analogs have attracted much attention as high affinity and selectivity ligands for the gastrin-releasing peptide (GRP) receptor. The GRP receptor was found to be overexpressed and implicated in a variety of human tumors. Radiolabeled bombesin and bombesin analogs represent an interesting class of diagnostic probes for molecular imaging of GRP receptor-expressing prostate cancer. This work is aimed at the development of an ^{18}F-labeled bombesin analog for molecular imaging GRP receptors in prostate cancer xenografts.

Methods: Aminopentanoic acid-modified bombesin analog BX-06053011 was conjugated with the bifunctional labeling agent N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) in borate buffer (pH = 8.2) for 30 min at 50 °C to give the desired ^{18}F-labeled bombesin analog [^{18}F]BX-00374436 (Fig. 1). Tumor-targeting of radiolabeled bombesin analog [^{18}F]BX-00374436 was evaluated in male nude mice bearing human prostate cancer (PC3) by means of biodistribution and dynamic small animal PET studies.

Results: ^{18}F-labeled bombesin analog [^{18}F]BX-00374436 was prepared in 30% radiochemical yield (based upon [^{18}F]SFB) within 80 min including HPLC purification, evaporation of HPLC eluent and formulation in 0.9% saline. The radiochemical purity exceeded 95%, and the specific activity was 20 GBq/μmol. The binding affinity (K_D) of fluorobenzoylated bombesin BX-00374436 was determined to be 0.7 nM. Radiotracer [^{18}F]BX-00374436 showed reasonable metabolic stability in mouse blood, being 65% of intact radiolabeled peptide after 60 min. Tumor uptake of [^{18}F]BX-00374436 in PC3 tumor bearing nude mice was 2.75 %ID/g after 5 min p.i., and 2.45 %ID/g after 60 min p.i. The receptor specificity of radiotracer [^{18}F]BX-00374436 could be demonstrated by effective blocking of tumor uptake in the presence of non-radioactive BX-00374436. Dynamic small animal PET imaging confirmed specific radiotracer uptake in the PC3 tumor.

Conclusions: The present study showed that ^{18}F-labeled bombesin analog [^{18}F]BX-00374436 is a suitable radiotracer for molecular imaging of GRP receptor-positive prostate cancer by means of PET.

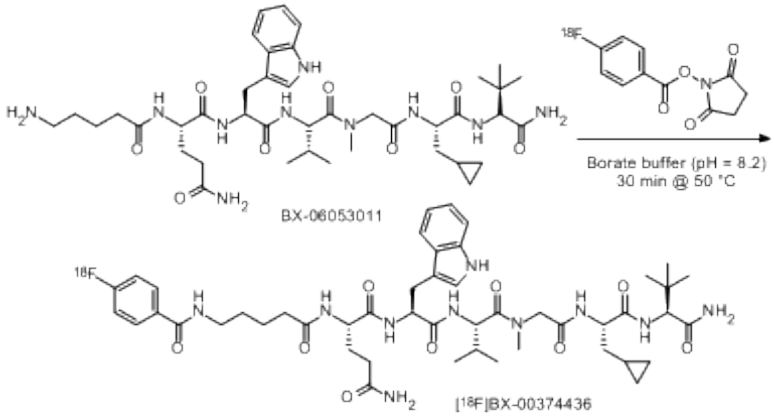

Fig. 1: Radiolabeling of bombesin analog BX-06053011 with [^{18}F]SFB

INVESTIGATION OF A NEW BOMBESIN DERIVATIVE FOR THE MOLECULAR IMAGING OF PROSTATE, BREAST, AND PANCREATIC CANCERS

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Objectives: The Bombesin analogue, DOTA-8-AOC-[(D)W^6]BBN(6-13)NHC$_2$H$_5$, was evaluated as a potential new targeting vector for ^{67}Ga/^{68}Ga molecular imaging of BB2 receptor (BB2r) expression using preclinical xenograft models of human prostate, breast, and pancreatic cancers.

Methods: ^{67}Ga- DOTA-8-AOC-[(D)W^6]BBN(6-13)NHC$_2$H$_5$ was synthesized and purified by HPLC prior to utilization. In vitro competitive binding assays were performed in PC-3 human prostate, T47D human breast, and CF-PAC1 human pancreatic tumor cell lines. In vivo pharmacokinetic studies were performed in SCID mice bearing bi-lateral PC-3, T47D, and CF-PAC1 human cell line flank tumor xenografts at 4-6 weeks post tumor cell inoculation. Tissues were collected at 15 min, 1, 4 and 24 hours P.I. of the ^{67}Ga conjugate. Correlative Micro-SPECT images of ^{67}Ga distribution were obtained at 4 hours P.I. in all three tumor xenograft SCID mice models.

Results: The ^{67}Ga conjugate showed rapid clearance from non-target tissues with tumor retention visualized in PC-3 prostate, T47-D breast, and CF-PAC1 pancreatic tumor xenografts when imaged using Micro-SPECT. Pharmacokinetic results demonstrated uptake in BB2r expressing tumor xenografts and the normal pancreas. The overall tumor to pancreas ratios at 4 hours P.I. remained high ranging from a value of 19:1 observed for the PC-3 prostate cancer xenograft model down to a 4:1 ratio observed in the CF-PAC1 pancreatic cancer xenograft model. Primary clearance of the ^{67}Ga conjugate was via the renal system with very limited renal retention observed. Variation in tumor uptake between cell lines studied corresponded with variations in BB2r expression for the respective cell line.

Conclusions: The pharmacokinetic and imaging properties of ^{67}Ga-DOTA-8-AOC-[(D)W^6]BBN(6-13)NHC$_2$H$_5$ demonstrate significant potential of this analog as a SPECT or PET molecular imaging agent to assess in vivo BB2r expression in a variety of cancers.

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IN VIVO EVALUATION OF [18F]FBA-FALGEA-NH2 AS A PET TRACER FOR EGFRvIII IMAGING

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Objectives: Early diagnosis and staging is of critical importance for correct treatment of cancer. Therfore, the identification of novel, cancer specific targets and receptor ligands for PET imaging is of great interest. This study describes the in vivo evaluation of H-FALGEA-NH_2 as a potential new tracer for imaging of the cancer specific epidermal growth factor receptor variant III mutation, EFGRvIII, using PET.

Methods: H-FALGEA-NH_2 was designed using a positional scanning synthetic combinatorial library (PS-SCL) using Fmoc chemistry [1]. The library consisted of six individual positional sub-libraries in the format, H-$O_{1\text{-}6}$XXXXX-NH_2, O being one of the 19 proteinogenic amino acids (cysteine omitted) and X an equimolar mixture of these. The binding of the peptide mixtures was tested using a biotin-streptavidin assay. For PET imaging H-FALGEA-$NH_2$was radiolabelled using 4-[^{18}F]fluorobenzoic acid [2]. The resin-bound peptide was radiolabelled by acylation with [^{18}F]FBA activated as an ester. The [^{18}F]-labelled peptide was then cleaved from the resin and purified using preparative HPLC. The in vivo studies of [^{18}F]FBA-FALGEA-NH_2 were performed on a MicroPET Focus 120 scanner, using nude mice (n=11) xenografted subcutaneously with human glioblastoma multiform tumour, expressing the mutated receptor in this native form. The mice were injected with 5-10 MBq of the radiolabelled peptide.

Results: The pure radiolabelled peptide was produced with in 180 min., in overall radiochemical yields of 2.6-9.8 % (decay-corrected) with a average specific radioactivity of 6.4 GBq/μmol. In the nude mice, [^{18}F]FBA-FALGEA-NH_2 accumulated in the human cancer xenografts. The tumour-to-muscle ratio (T/M) was up to 30 (average 7.8) and SUV 0.08-0.17 at 60 min post injection and T/M up to 15 (average 4.5) at 240 min, indicating selective uptake of the radiolabelled peptide in the tumours.

Conclusions: In conclusion, our results show that [^{18}F]FBA-FALGEA-NH_2 is a promising ligand for PET imaging of EGFRvIII.

Reference: [1] Denholt, C.L., Hansen P.R., Pedersen, N., Poulsen, H.S., Gillings, N., Kjær, A. Identification of novel peptide ligands for the cancer-specific mutation EFGRvIII using a mixture-based synthetic combinatorial library. Biopolymers, 2009. 91,:201 – 206 [2] Sutcliffe-Goulden, J.L., O'Doherty, M.J., Marsden, P.K., Hart, I.R., Marshall, J.F., Bansal, S.S., Rapid solid phase synthesis and biodistribution of ^{18}F-labelled linear peptides. Eur. J. Nucl. Med. Mol. Imaging, 2002. 29:754-759.

Research Support: The Danish Cancer Society for fundings.

RADIOSYNTHESIS AND BIODISTRIBUTION OF CYCLIC RGD PEPTIDES CONJUGATED WITH A NOVEL [18F] FLUORINATED N-METHYLAMINOOXY CONTAINING PROSTHETIC GROUP

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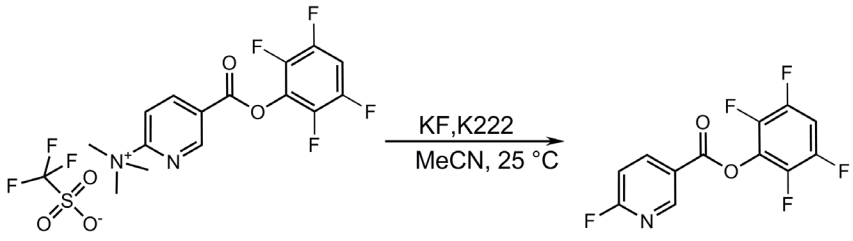
Objectives: We have recently reported the use of an ^{18}F-prosthetic group based on the reactivity of the N-methylaminooxy group with peptides modified with strong Michael-acceptors. The conjugation is done in mildly acidic aqueous solution. Here we extend our studies by applying this chemistry for labelling of cyclic RGD peptides. The ^{18}F-labeled peptides were subjected to MicroPET and biodistribution studies in xenograft bearing mice.



Scheme 1. Synthesis of the two [^{18}F]-RGD peptides by conjugation with the [^{18}F]-N-methylaminooxy prosthetic group.

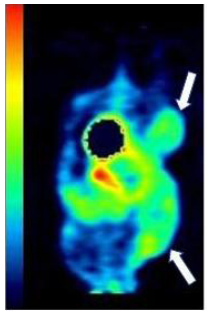
Methods: Modified cyclic RGD peptides were synthesised from the peptide intermediate NC100717 [1]. A dicysteic acid moiety was attached to the free ε-amino group of lysine, to give satisfactory water solubility for the conjugation reaction and also render the conjugate more hydrophilic and the possibility of more favourable excretion pattern. Two version of peptide were synthesised either with 3-ethensulfonyl propionic acid or 4-(2-Nitrovinyl)benzoic acid as Michael acceptors for reaction with the [^{18}F]-N-methylaminooxy prosthetic group. ^{19}F-labeled peptides were tested in competition with ^{125}I-echistatin with EA-Hy926 membranes to determine Ki values. Radiosynthesis: Both [^{18}F]-peptides were synthesis using a commercial platform. After purification by reversed HPLC, [^{18}F]-conjugates were formulated in saline (100 MBq/mL). Biodistribution and MicroPET studies: Nude mice with s.c. osteosarcroma (OHS) cells were allowed to develop for 2 weeks followed by injection via the tail vein with 5-8 MBq of [^{18}F]-peptide. After CT and 120 min dynamic MicroPET scan the mice were sacrificed and major organs weighed and counted.

Results: Both peptides displayed Ki values bellow 10 nM. [^{18}F]-peptides were synthesised in 6-12 % decay corrected yield based on starting amount of [^{18}F]fluoride with a RCP greater than 98%. The [^{18}F]-4-(2-Nitrovinyl)benzoyl peptide showed poor in vitro plasma stability and was thus not evaluated further. [^{18}F]-3-ethensulfonyl propionyl RGD peptide displayed excellent plasma stability. MicroPET imaging studies reveal that the compound is taken up in the tumour, with good tumour to background ratios (1.5 from microPET). Also high uptake was observed in liver. The compound predominantly clears through urine. No signs of defluroination were observed.

Conclusions: [^{18}F]-3-ethensulfonyl propionyl RGD peptide is a useful [^{18}F]-peptide for imaging integrin $\alpha_v\beta_3/\alpha_v\beta_5$. MicroPET and biodistribution studies indicate higher uptake in tumour compared to background 120 min p.i. Tumours are clearly visualized in MicroPET images. [^{18}F]-4-(2-Nitrovinyl)benzoyl displayed too poor plasma stability for in vivo studies.

References: 1. Indrevoll et al, NC100717: A versatile RGD peptide scaffold for angiogenesis imaging. Bioorganic & Medicinal Chemistry Letters 16, p. 6190-6193 (2006)

Figure 1. Dynamic MicroPET scan of tumour bearing mice 100 min post injection. Two tumours can be visualised on the right side (indicated by white arrows).



IN VITRO AND IN VIVO EVALUATION OF STRUCTURALLY DIVERSE CU-64-LABELED RGD PEPTIDES FOR PET IMAGING OF αVβ3 EXPRESSION

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Objectives: Integrin $\alpha_v\beta_3$ is upregulated in tumor vasculature, osteoclasts and areas of collateral circulation following ischemic injury. Here, we investigate structurally diverse bifunctional RGD (arginine-glycine-aspartic acid) peptides for $\alpha_v\beta_3$ integrin affinity in vitro and in vivo. Bifunctional ligands allow for targeting with a peptide as well as radiolabeling with ^{64}Cu via a macrocyclic chelator for PET imaging.

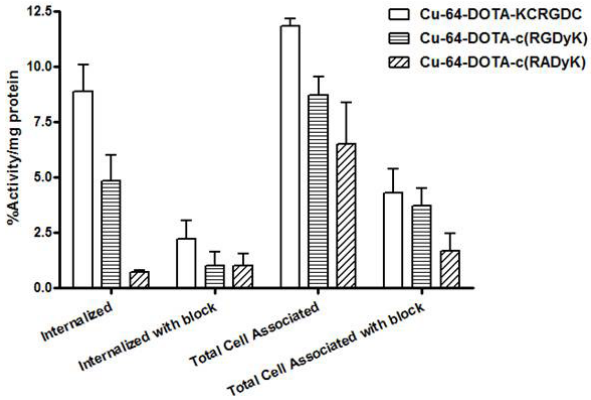
Methods: A series of peptides containing the RGD sequence were synthesized. Affinity to $\alpha_v\beta_3$ and specificity for $\alpha_v\beta_5$ and $\alpha_{IIb}\beta_3$ were determined in an isolated, competitive binding assay. Cellular uptake studies in an $\alpha_v\beta_3$-positive cell line (U87MG human glioblastoma cells) were performed using the ^{64}Cu-DOTA-labeled peptides. In vivo characteristics were examined in biodistribution and microPET studies in U87MG tumor-bearing nu/nu mice.

Results: A lactam-cyclized peptide (c(RGDyK) demonstrated higher affinity for $\alpha_v\beta_3$ than disulfide-cyclized (KCRGDC) or linear peptide (GRGDS) (Table 1). Disulfide-cyclized peptides exhibit the best selectivity of the ligands investigated. A control, lactam-cyclized peptide (c(RADyK)) has low affinity for all three integrins. Conjugation of Cu(II)-containing chelators did not affect the binding affinity or selectivity of the peptides.

Table 1. IC_{50} values for RGD peptides.

	αvβ3 (nM)	αvβ5 (nM)	αIIbβ3 (nM)
c(RGDyK)	3.7	171	0.11
KCRGDC	10.4	921	>5,000
GRGDS	15.9	>5,000	873
c(RADyK)	1,400	>5,000	>5,000

In cell studies the disulfide-cyclized peptide showed higher internalization than the lactam-cyclized peptide (Figure 1).



Uptake of RGD peptides could be blocked by high doses of unlabeled peptide. Internalization of the control peptide was minimal.

Biodistribution studies in the murine tumor model showed rapid uptake and retention of the ^{64}Cu-labeled peptides in tumor with good tumor:blood and tumor:muscle ratios (^{64}Cu-DOTA-KCRGDC, 4h post-injection: 4.23 ± 1.4 and 8.56 ± 2.4 respectively). Uptake can be blocked by co-administration of high doses of unlabeled peptide.

Conclusions: The peptides will be further investigated via microPET imaging of $\alpha_v\beta_3$-expressing animal models, including the U87MG tumor model and hind limb ischemia for imaging angiogenesis following ischemic injury. In addition to evaluating the radiolabeled $\alpha_v\beta_3$-targeted peptides, we are also pursuing the goal of accomplishing higher binding affinity by attaching several peptides onto nanoparticles.

VPAC1 GENE PRODUCT TARGETED PET IMAGING OF BREAST CANCER: FROM RODENTS TO HUMANS

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Objectives: Among women in the USA, breast cancer (BC) accounts for more than 25% of all cancers. Although mammography detects advanced lumps, it misses small lesions, particularly in young women and fails to stratify benign or malignant status of the disease. Early and accurate diagnosis can abate morbidity and mortality. All human BCs overexpress VPAC1 gene product. The objective was to target VPAC1 receptors with a specific biomolecules labeled with positron emitting radionuclide will provide an effective approach for early imaging of BC and stratify its malignancy.

Methods: Four peptides with high affinity for VPAC1 were chosen, synthesized, modified to chelate Cu-64, purified, and characterized using mass spectrometry, muscle relaxivity, and cell binding assays. Diaminedithiol (N_2S_2) served as a chelating agent. Tissue distribution, receptor blocking blood clearance and PET imaging studies were performed. MCF-7 human BC xenografts in athymic nude and transgenic MMTV nue (mouse mammary tumor virus) mice were used as animal models. Toxicology studies were performed and estimated radiation dose was calculated. Human BC tissue autoradiography and RT-PCR studies were carried out. eIND was filed and IRB approval was obtained. Human studies were initiated in which F-18-FDG served as gold standard.

Results: Cu-64 labeling efficiency for the 4 analogs ranged between 94.6±5.8 and 97.5±4%. Ic50 values were between 4.4 x 10^{-8} M and 8.1 x 10^{-8} M and Kd values between 0.2 X 10^{-9}M and 3.3 x 10^{-9}M. Blood half clearance time was ~ 3 min (range 3.1-3.3min) for all analogs with β half clearance time ranging between 45 and 160min. Blocking studies showed high receptor specificity and in vivo stability was excellent. Four hr tumor uptake was 5% through 6.8%ID/g and the 24hr values were 5% through 10.3% ±2.4%IDG. In MMTV mice, occult tumors that were not detectable with F-18-FDG were visualized by Cu-64-TP3805, one of the 4 peptides. On all tumors, VPAC1 receptors were expressed. No toxic events were noted and radiation dosemetry was considered acceptable. At the time of this writing, one patient has been enrolled for PET imaging with Cu-64-TP3805. Consistent with F-18-FDG results, a small primary tumor in the right breast and a lymph node was visualized with Cu-64-TP3805 PET scan. Renal, bladder and lung uptake was negligible.

Conclusions: Four peptides labeled with Cu-64 have been evaluated to determine their ability to target gene product VPAC1 abundantly expressed on all human BC. Results, ex vivo, in nude mice with experimental human BC, in MMTV nue transgenic mice and in one human case with BC studied thus far have been highly encouraging.

Research Support: This work was supported by NIH grant CA-109231, EB-001809, RP-023709 and to PA department of health funding Grant No ME-03-184.

64Cu LABELED CHLOROTOXIN AS A POTENTIAL IMAGING AGENT FOR GLIOMA

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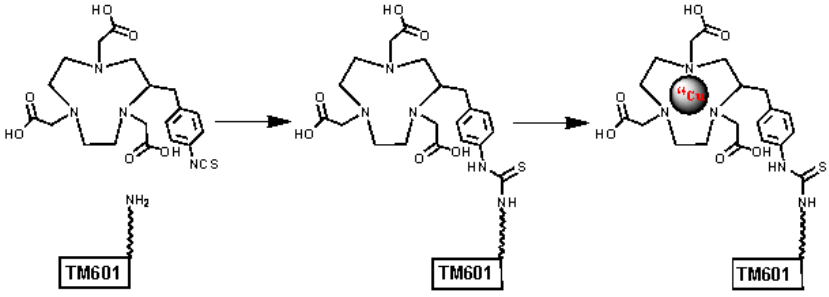
Objectives: TM601, a synthetic version of chlorotoxin, a 36 amino acid small peptide originally isolated from the giant Israeli scorpion[1] has been shown to have high uptake in gliomas, and its ^{131}I derivative has shown promising results in Phase II therapy and imaging trials.[2] A fluorescent analog of TM601 is being evaluated as an intraoperative visualization agent for cancer foci to improve detection and surgical resection of tumors.[3] Iron oxide nanoparticles functionalized with chlorotoxin have shown promising results both as imaging and therapeutic agents.[4] As an alternative to ^{131}I for imaging and potentially therapy of tumors that internalize TM601, we synthesized a NOTA analog of this peptide and labeled it with ^{64}Cu ($T_{1/2}$ = 12.7 h; ß$^+$ (17.8%)).

Methods: The new compound was synthesized, purified and radiolabeled with ^{64}Cu. Cellular internalization experiments were performed with ^{64}Cu-NOTA-TM601 using adherent U87MG, 9L, PC3, MCF-7, and NHDF cell lines. The cells were incubated with 4 nM ^{64}Cu-NOTA-TM601 with or without 250-fold excess of competing TM601 for 1 hour at 37°C. Cells were washed, stripped with trypsin to determine internalization, and counted in a γ-counter. MicroPET imaging studies were performed on rats bearing tumors (U87MG) followed by biodistribution analysis.

Results: TM601-NOTA was synthesized and purified by HPLC. TM601 has three lysines and the N-terminal group with potential reactivity with the isothiocyanate group in the NOTA derivative; however, under the conditions used for coupling, TM601 molecules preferentially formed the mono-NOTA macrocycle derivative.



^{64}Cu-NOTA-TM601 was readily labeled with a specific activity of 150 mCi/mg. This complex was stable in rat serum at 37° C for 24 hours. There are several cell lines that show specific affinity for the peptide, including U87MG (human glioma), PC-3 (human prostate cancer) and 9L (rat brain tumor). Tumor cell binding has been shown to require Annexin A2, a Ca^{2+}-dependent membrane binding protein. NHDF (human dermal fibroblasts) and MCF-7 (human breast adenocarcinoma) cells do not have detectable affinity for chlorotoxin. Cellular internalization studies showed that U87MG, 9L, and PC3 cells internalized the tracer (50-60%) and internalization was significantly blocked with excess non-labeled TM601 suggesting a receptor-specific process. MCF-7 and NHDF cells showed no specific internalization. Finally, biodistribution and microPET imaging with ^{64}Cu-TM601-NOTA were performed in tumor-bearing nude mice implanted with U87MG cells. Although there was high uptake in the kidneys, the tumor:blood and tumor:muscle ratios were 5.9±1.1 and 10.1±4.2, respectively.

Conclusions: TM601-NOTA was successfully radiolabeled with ^{64}Cu and used to image tumors in rats. Research is underway to investigate whether conjugation of TM601 to different chelators can improve the biodistribution profile and lower kidney uptake.

Research Support: Funding for this research was provided in part by TransMolecular, Inc.

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