ASSESSMENT OF NATRIURETIC PEPTIDE CLEARANCE RECEPTOR WITH POSITRON EMISSION TOMOGRAPHY IN HINDLIMB ISCHEMIA MODEL

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Objectives: Pathological angiogenesis is a hallmark of cancerous tumor development and various ischemic and inflammatory diseases. Molecular imaging of angiogenesis would therefore be helpful in the recognition and treatment of angiogenesis associated disease. Natriuretic peptides (NPs) are a group of peptide hormones that maintain the health of the cardiovascular system. Among the three NPs, C-type NP is derived primarily from endothelial cells and acts as a potent inhibitor of vascular smooth muscle cell migration and proliferation through activation of the clearance receptor (NPR-C). In this study, a Cu-64 labeled NP fragment was employed as a probe for non-invasive imaging with PET of NPR-C receptor expression in an animal model of angiogenesis.

Methods: Hindlimb ischemia, which has been shown to stimulate angiogenesis, was induced in male C57BL/6 mice by ligation and excision of a segment of the right femoral artery. A sham surgery was performed on the left femoral as a control. Blood flow assessed with a deep muscle Doppler probe and O-15 water PET imaging were used to verify the presence of ischemia immediately after excision of the femoral segment and the increased blood flow induced by angiogenesis 1 week later. CANF (atrial natriuretic factor), which is a C-type NP truncated analog, was functionalized with DOTA and labeled with Cu-64for imaging studies. One week after induction of ischemia, a microCAT scan was performed to collect anatomic information and then ^{64}Cu-DOTA-CANF (0.74 MBq, 18.8 pmol CANF peptide) was injected I.V. and a 1 h dynamic scan was collected on the microPET Focus-220. Fiducial markers were attached to the scanner bed for PET/CT co-registration. Histopathologic imaging and immunohistochemistry (IHC) of the ex vivo thigh muscle were performed to confirm the presence of NPR-C receptors. PET imaging after I.V. co-injection of CANF peptide as a blocking agent with ^{64}Cu-DOTA-CANF (100:1 mole ratio) was performed to prove the receptor mediated tracer uptake.

Results: MicroPET imaging clearly showed the tracer uptake in the injured thigh muscle with virtually no uptake observed in the control thigh muscle. The standard uptake value (SUV) ratio of injury/control thigh muscle was 2.34±0.41 (n=4). In the blocking study, the injury/control SUV ratio was 1.24±0.26 (n=4), significantly less than the results without blocking ($p<0.005$). In addition, the IHC images also proved the presence of NPR-C receptors in the injured thigh muscle and no receptor in the sham-operated muscle. Both PET and IHC showed receptor specific uptake in the injured thigh.

Conclusions: This study shows that NPR-C receptors are upregulated in the ischemic hindlimb of mice consistent with induction of angiogenesis. The results also show that ^{64}Cu-DOTA-CANF is a promising candidate tracer for molecular imaging of NPR-C receptors in the ischemia induced angiogenesis model.

Research Support: This work is supported by the NIH as a Program of Excellence in Nanotechnology (HL080729). The production of 64-Cu is supported by a NCI grant (CA86307).

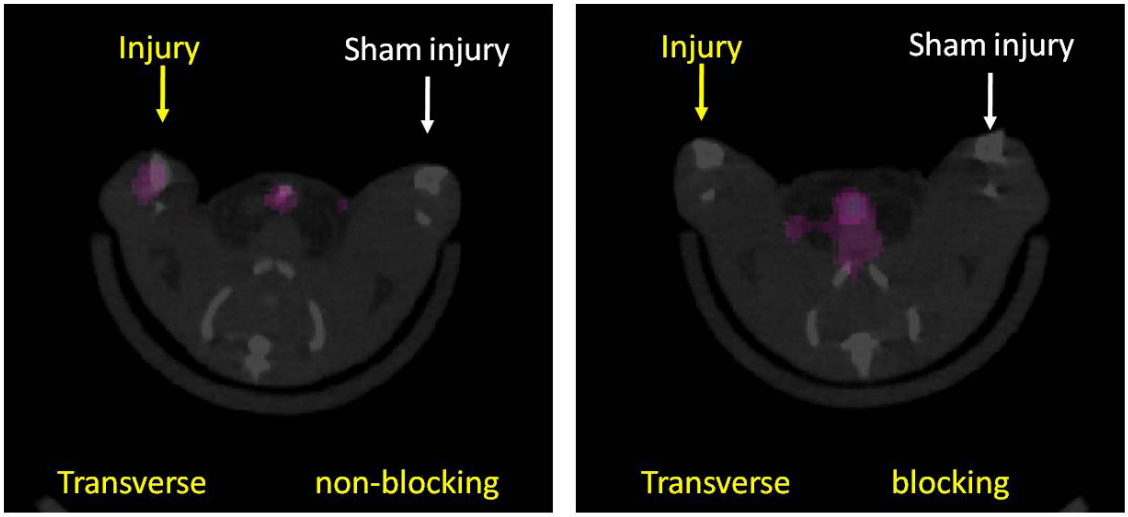

Figure 1. ^{64}Cu-DOTA-CANF tracer uptake at injury and sham injury

64Cu-AMD3100 – A NOVEL IMAGING AGENT FOR TARGETING CHEMOKINE RECEPTOR CXCR4 POSITIVE TUMORS

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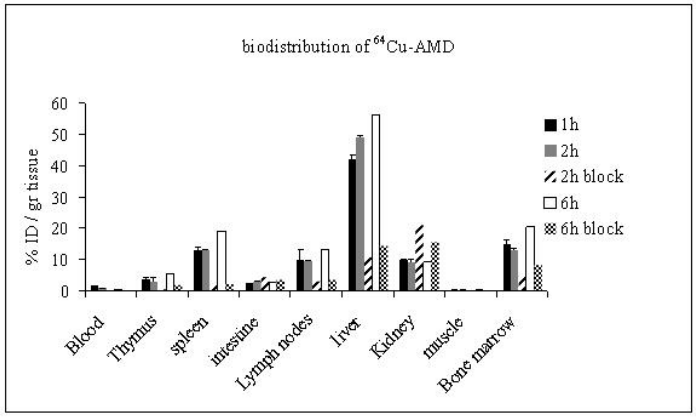
Objectives: More than twenty-three different human tumors, including breast, prostate, lung, ovarian, pancreatic, esophageal, colorectal, and renal carcinoma, melanoma, neuroblastoma, and osteosarcoma, over-express the chemokine receptor, CXCR4. CXCR4 has been shown to be exploited by various tumors for increased survival, invasion, proliferation, vascularization, metastasis and homing to target organs. While PET has the potential to visualize CXCR4 expression of tumor cells in vivo, fully optimized CXCR4 imaging agents are yet to be found. We have developed a specific labeled biomarker that enables in vivo quantification of CXCR4 in mouse xenograft tumors.

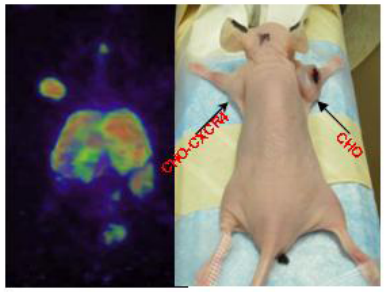
Methods: We developed a one step radiosynthesis for labeling the CXCR4-specific antagonist AMD3100 with Cu-64 to produce ^{64}Cu-AMD with a high specific activity.The affinity and specificity of the new tracer to CXCR4 were tested in vitro using human Jurkat T cells and mouse splenocytes. Athymic nude mice were inoculated subcutaneously with either CXCR4 transfected- or nontransfected tumor cells, to establish xenograft models. In vivo accumulation of ^{64}Cu-AMD was quantified using both biodistribution experiments and small animal PET scans.

Results: Incorporation of copper into AMD did not affect its binding or CXCR4 inhibitory properties. The biodistribution of ^{64}Cu-AMD was analyzed in immune competent C57BL/6 mice using both PET scans and gamma counting of dissected organs. Specific accumulation of ^{64}Cu-AMD was observed in CXCR4 expressing organs such as the spleen, lymph nodes and bone marrow, as well as accumulation in metabolic organs. Injection of ^{64}Cu-AMD to Athymic nude mice bearing CXCR4 positive and CXCR4 negative tumors enabled clear visualization of CXCR4 positive tumors but not CXCR4 negative tumors, within the first hour after injection, and increased over time. Moreover, blocking experiments with unlabeled Cu-AMD abolished the accumulation of the labeled ligand in the CXCR4 positive tumors, which indicates specific binding.

Conclusions: We conclude that ^{64}Cu-AMD can be used as an in vivo agent for imaging CXCR4 expression by tumors.



Biodistribution of ^{64}Cu-AMD in normal C57Bl/6 mice. In blocking experiments, the tracer was injected together with unlabeled Cu-AMD. Each group contain at least 5 mice. Results shown are average ±SE.



^{64}CuAMD was injected to a mouse bearing CHO (right) and CHO-CXCR4 (left) tumors, and imaged by PET scan. %ID/g = 15.7.

SYNTHESES AND PRELIMINARY APPLICATION OF 68GA-SCHIFF BASE DERIVATIVES FOR IN VIVO IMAGING OF THE P-GLYCOPROTEIN STATUS IN TUMOURS

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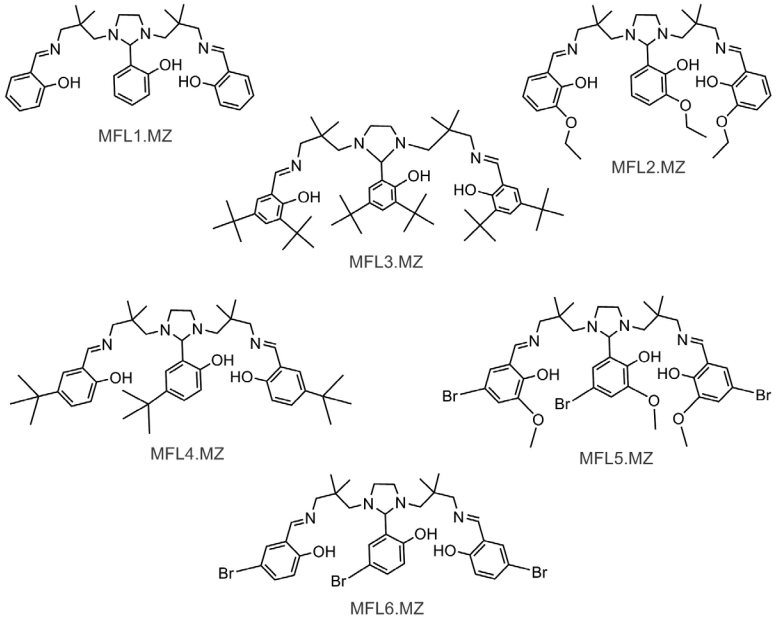
Objectives: Cells contain very effective mechanisms to transport xenobiotics out of the cell. Thereby the intracellular concentration of drugs is lowered (multidrug resistance). The most important of these ABC-transporters is p-glycoprotein (pGP), which transports neutral and cationic structures. In many tumours pGP is over expressed leading to a very low concentration of several chemotherapeutics. In order to identify multidrug resistant tumours, a PET-tracer would be helpful with the following features: (1) enter the cell easily (by passive diffusion) and (2) tracer should be a substrate of pGP and transport should be inhibitable by e.g. verapamil. Using the ^{68}Ge/^{68}Ga generator, novel ^{68}Ga-based Schiff base ligands provide interesting molecules accomplishing both requirements.

Methods: Based on a ligand by Sharma [1] six derivatives were synthesized. For labelling with a positron emitter the ^{68}Ge/^{68}Ga generator was used which provides Gallium-68 in 400 μL acetone/HCl mixture [2]. Labelling is performed in 400 μL 0.12 M Na-HEPES buffer by adding the ^{68}Ga fraction. Through variation of reaction time, temperature and different amounts of the ligands, optimum reaction parameters for complexation were tested. Cell studies on rat prostate carcinoma cells in presence or absence of verapamil where performed for all ligands to prove the transport ability by pGP.



The most interesting ligand MFL6.MZ was used for in vivo studies concerning uptake in solid growing rat tumours and compared to the reference ligand.

Results: Labelling proceeds at 25 to 75°C within 2 to 10 min. Ligands are used in nanomole amounts only and the radiochemical yields are 50 to 95%. Cell essays showed that beside one ligand all others were transported by pGP. However, the uptake into the cell by non-ionic diffusion varied broadly. The ligand MFL6.MZ was the most promising compound. Under normal conditions, 25% of the activity was in the cell. When inhibiting the pGP, it increased to 35%. Compared to the literature ligand (4.6 % not inhibited; 8.2 % inhibited) this ^{68}Ga complex was selected for in vivo studies on a μ-PET. Imaging ^{68}Ga-MFL6.MZ revealed a 3-fold higher accumulation in tumours compared to the reference tissue (testicles), whereas the literature compound is only slightly enriched in the tumour.

Conclusions: Six Schiff base ligands were synthesized and labelled with ^{68}Ga. Tumour cell studies showed uptake in cells and transport processes by pGP for five ligands. ^{68}Ga-MFL6.MZ was chosen for μ-PET imaging on tumour bearing rats demonstrating a high uptake in tumour.. Further studies will involve blocking pGP in vivo and raising transport activity of pGP. With ^{68}Ga-MFL6.MZ it appears to be possible to identify patients with multidrug-resistant tumours pre-therapeutically in order to select adequate treatment regimes.

References: [1] Sharma et al, J Nucl Med 46: 354-364 [2] Zhernosekov et al, J Nucl Med 48: 1741-1748

EVALUATION OF A [67Ga] THIOSEMICARBAZONE COMPLEX AS TUMOR IMAGING AGENT

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Objectives: Thiosemicarbazone gallium complexes have shown interesting anti-proliferative activity in vitro and in vivo. The most studied compounds are pyridine-based compounds.Due to the importance of pyridine thiosemicarbazones in anti-neoblastic activity and the necessity of gallium complexation in most of these compounds for enhancement of their activity, the idea of developing a possible tumor imaging agent using SPECT by incorporating ^{67}Ga into a suitable chelate, i.e. $APTSM_2$ was investigated.

Methods: No unlabelled and/or labeled by-products were observed by ITLC/HPLC analysis of the final preparations. The radio-labeled complex was stable in aqueous solution and in human serum at 37ºC, for at least 2 days, and no significant amount of other radioactive species were detected by ITLC. Biodistribution of the complex in fibrosarcoma bearing mice demonstrated significant tumor uptake at 2h post injection. The SPECT images also showed the specific accumulation in tumors at 2h post injection.

Results: [^{67}Ga]labeled 2-acetylpyridine 4,4-dimethyl thiosemicarbazone ([^{67}Ga][$APTSM_2$]$_2^+$) was prepared using freshly prepared [^{67}Ga]$GaCl_3$ and 2-acetylpyridine 4,4-dimethyl thiosemicarbazone ($APTSM_2$) for 60 min at 90°C (radiochemical purity: >95% ITLC, >98% HPLC). Stability of the complex was checked in human serum for 37ºC. The biodistribution of the labeled compound in vital organs of normal and fibrosarcoma bearing mice were compared with that of free Ga^{3+} cation rats up to 24h.

Conclusions: All the tests were compared with that of $^{67}GaCl_3$ in tumor bearing rodents showing the different pattern of accumulation of the tracer. It is suggested that [^{67}Ga][$APTSM_2$]$_2^+$ could be a possible SPECT tracer, however considering the fast tumor uptake, the short half life gallium-68 can be a better candidate for tumor imaging applications and future [^{68}Ga]-PET studies and less imposed radiation doses to patients.

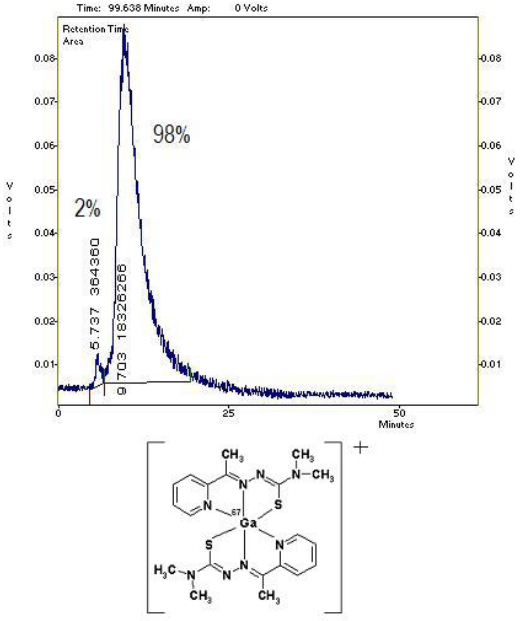

Fig. 1. Structure of [^{67}Ga][$APTSM_2$]$_2$+

NOVEL DIMERIC GALLIUM-68 LABELED TYROSINE-DERIVATIVES – POTENTIAL TUMOR TRACERS FOR PET

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Objectives: Some radiolabeled amino acids such as [^{11}C-methyl]-L-methionine ([^{11}C]MET) [1], 2-[^{18}F]-L-tyrosine [2] or 2-O-[^{18}F]fluoroethyl-L-tyrosine ([^{18}F]FET) [3] are well established as metabolic imaging tracers for positron emission tomography (PET). ^{68}Ge/^{68}Ga-generator produced ^{68}Ga is an upcoming PET nuclide because of its availability and comparably low costs. Consequently, it is a challenge to combine the advantages of labeled amino acids and ^{68}Ga as radionuclide to develop a suitable ^{68}Ga-labeled amino acid for PET/CT tumor imaging. We chose 1,4,7,10-tetraazacyclododecane-1,7-diacetate (DO2A) and 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (DO3A) as bifunctional chelators for ^{68}Ga(III) and tyrosine as amino acid targeting vector.

Methods: The bifunctional chelators were synthesized following a modified method of Kovacs and Sherry starting from cyclen [4]. Different 1-bromo-n-chloro compounds were used as spacer. These were coupled in a nucleophilic substitution to the phenolic hydroxyl group of N-BOC- and O-Me-protected tyrosine. One (DO2A and DO3A) or two (DO2A) of these spacer-tyrosine moieties were attached to the chelator. After cleavage of the protective groups (NaOH (1M)/dioxane, TFA), ^{68}Ga-labeling was carried out in water (5 ml) with the labeling precursor (10 μg) at 90°C [5]. Radiochemical yields and purity were determined by radio-HPLC and radio-TLC. In-vitro studies were performed using the rat F98 glioma cell line (ATCC, CRL-2397).

Results: The ^{68}Ga-labeled products were obtained in up to 95% yield after 10 min and a radiochemical purity of greater than 99 % after solid phase extraction. High stability of the ^{68}Ga-labeled product was observed in the DTPA-challenge experiment in vitro. Uptake of [^{68}Ga]DO2A-(butyl-L-tyrosine)$_2$ in F98 cells was measured at 0°C, when non-specific uptake was expressed as the uptake of [^{68}Ga]DO2A alone. The uptake of [^{68}Ga]DO2A-(butyl-L-tyrosine)$_2$ was significantly blocked by a mixture of BCH, Trp and Ser), reaching the level of non-specific uptake (Fig. 1). These studies indicate specific uptake of [^{68}Ga]DO2A-(butyl-L-tyrosine)$_2$ into F98 cells mediated by an amino acid transporter.

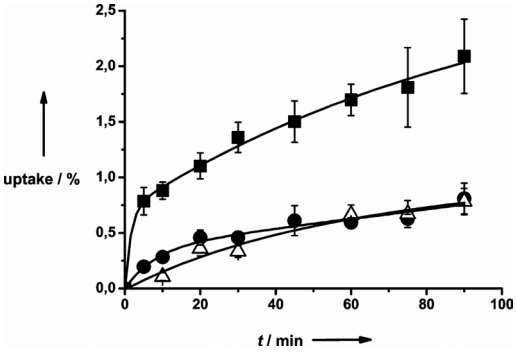

Figure1: a) Total uptake of ^{68}Ga-labeled DO2A-(butyl-L-tyrosine)$_2$ in F-98-glioblastoma cells, (squares, n=6) b) Non-specific uptake measured as uptake of ^{68}Ga-DO2A, (circles, n=6), c) uptake in the presence of an amino acid cocktail (Trp, Ser, BCH), (triangles, n=3)

Conclusions: Further studies are needed to characterize the molecular mechanisms governing uptake of [^{68}Ga]DO2A-(butyl-L-tyrosine)$_2$ in F98 cells. Evaluation of a series of alternative dimeric candidates started, in order to examine the influence of the spacer and the number of tyrosines on the cell uptake. μPET-studies with the most promising candidates will be performed using F-98-bearing rats.

References: [1] B. Langstrm, G. Antoni, P. Gullberg, C. Halldin, P. Malmborg, K. Nagren, A. Rimland, H. Svrd, J. Nucl. Med. 28 (1987) 1037. [2] H. H. Coenen, P. Kling, G. Stcklin, J. Nucl. Med. 30 (1989) 1367. [3] H. J. Wester, M. Herz, W. Weber, P. Heiss, R. Senekowitsch-Schmidtke, M. Schwaiger, G. Stcklin, J. Nucl. Med. 40 (1999) 205. [4]Z. Kovacs, A. D. Sherry, J. Chem. Soc., Chem. Commun. (1995) 185. [5] K. Zhernosekov, D. Filosofov, R. P. Baum, P. Aschoff, H. Bihl, A. A. Razbash, M. Jahn, M. Jennewein, F. Roesch, J. Nucl. Med. 48 (2007) 1741.

[99mTc]TRICARBONYL-LABELED PSMA INHIBITORS FOR PROSTATE CANCER IMAGING

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Objectives: Prostate specific membrane antigen (PSMA) is a type II integral membrane protein that has abundant expression on the surface of prostate cancer cells, particularly in androgen-independent, advanced and metastatic disease. Previously we reported the synthesis and evaluation of a series of [^{99m}Tc]tricarbonyl-labeled PSMA inhibitors, where compound L1 (Figure 1) demonstrated specific uptake in PSMA+ tumor xenografts.[1] In order to investigate further the effect of different [^{99m}Tc]tricarbonyl chelates in this system on tumor uptake and pharmacokinetics we have synthesized three additional hydrophilic ligands, (SRB1 = $(NH_2)CH(CO_2H)CH_2$-triazole-$(CH_2)_4CH(CO_2H)$-NHCO-$(CH_2)_6$CO-NH-urea, SRB2 = Bis-imidazolyl-$N(CH_2)_4CH(CO_2H)$-NHCO-$(CH_2)_6$CO-NH-urea and SRB3 = monopyridylmonoacid-$N(CH_2)_4CH(CO_2H)$-NHCO-$(CH_2)_6$CO-NH-urea respectively for radiolabeling with [^{99m}Tc]tricarbonyl.

Methods: Syntheses of the radioligands are shown in Figure 1. Compound SRB1 was prepared using click chemistry.[2] The three compounds and their corresponding rhenium complexes were characterized using standard spectroscopic techniques. A fluorescence-based NAALADase assay[1] was used to determine the K_i values of these new compounds. SPECT-CT imaging was performed using either a SCID mouse bearing a PSMA+ LNCaP tumor or using a SCID mouse bearing a PSMA+ PC-3 PIP and PSMA- PC-3 flu tumors on either shoulder. Animals were administered 1 mCi (37 MBq) of each agent intravenously, and were imaged on the XSPECT scanner.

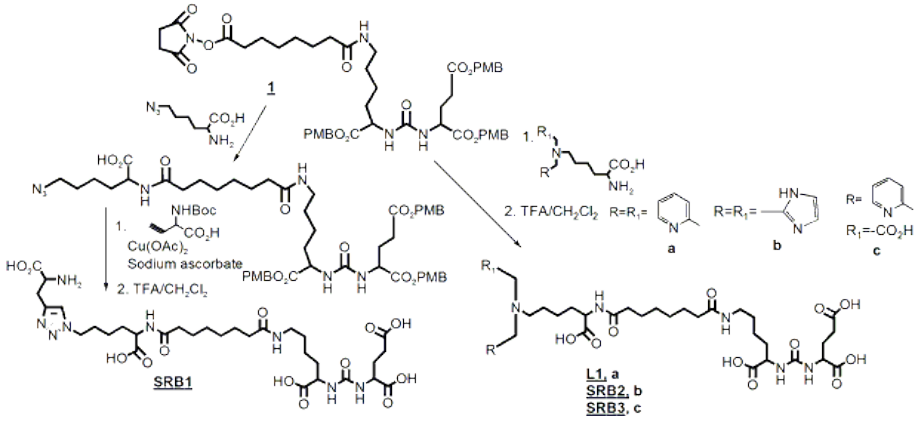
Results: These radioligands were generated in high radiochemical yields (70% - 95%) and purities (> 98 %), using a [^{99m}Tc] tricarbonyl labeling kit. The PSMA inhibitory capacity (K_i values) of the compounds and the corresponding rhenium complexes are in the range of 3.8 nM to 30 nM. SPECT-CT imaging studies revealed that [^{99m}Tc]SRB1 and [^{99m}Tc]SRB3 demonstrated site-specific uptake in PSMA+ tumors. [^{99m}Tc]SRB1 showed superior pharmacokinetics compared to our previous ^{99m}Tc-labeled ligands, i.e., higher tumor uptake, lower GI and liver uptake at 30 min postinjection, and faster clearance from most nontarget organs at 3.5 h p.i. No uptake was found in PSMA- tumors. [^{99m}Tc]SRB2 showed relatively high GI, liver and gall bladder uptake at 30 min p.i. Ex vivo biodistribution studies of these agents are in progress and will be presented.

Conclusions: Both [^{99m}Tc]SRB1 and [^{99m}Tc]SRB3 localized specifically to PSMA+ tumor xenografts. [^{99m}Tc]SRB1 clears rapidly from most normal organs (except kidneys, a PSMA+ organ) due to its hydrophilic nature, resulting in high tumor/organ ratios, and is a promising candidate for SPECT imaging of prostate cancer.

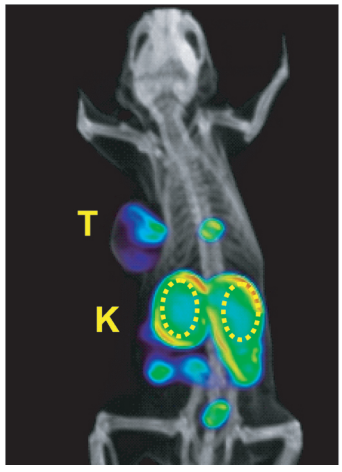
Research Support: This work was supported by the following grants: NIH R24CA92871, NIH R21 CA114111, and DoD PC050999

References: 1. J Med Chem 2008; 51:4504-4517. 2. Bioconjugate Chem. 2008;19:1689-1695

Figure 1



SPECT-CT image of [Tc-99m]-SRB1 at 3.5 h post injection using PSMA+ LnCaP tumor xenograft in a male SCID mouse



EFFECT OF CHARGE ON UPTAKE AND RETENTION OF 2-NITROIMIDAZOLE-99mTc(CO)3 COMPLEXES IN HYPOXIC TUMORS

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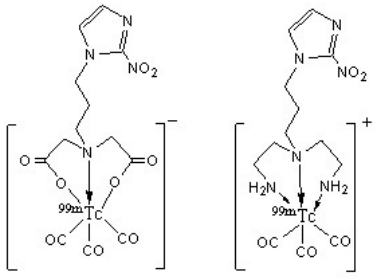
Objectives: Hypoxia plays a major role in contributing towards the resistance of tumors to both radiation therapy and chemotherapy. The uptake and retention of a nitroimidazole in hypoxic tumors is decided by a combination of different factors like lipophilicity, charge on the complex and its single electron reduction potential. Radiolabeling of nitroimidazole derivatives, particularly 2-nitroimidazoles has been attempted with varying degrees of success using ^{99m}Tc. Herein, we report the syntheses and in vivo evaluation of two differently charged nitroimidazole derivatives in Swiss mice bearing fibrosarcoma.

Methods: The two ligands, an iminodiacetic acid derivative (IDA) and a diethylenetriamine derivative (DETA) of 2-nitroimidazole were obtained via covalent conjugations of 2-nitroimidazole with pre-synthesized bifunctional chelating agents viz. N,N-bis[(tert-butoxycarbonyl)methyl]-3-bromopropylamine and N,N-bis[(N-phthaloylethyl)]-3-bromopropylamine respectively. Hydrolysis of the tert-butyl ester derivative and the deprotection of phthalimidodiethylenetriamine derivative formed as intermediates, yielded the target ligands. The 2-nitroimidazole-BFCA conjugates were labeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core, and subsequently characterized by HPLC. As per the envisaged strategy, 2-nitroimidazole was synthetically tailored so as to lead to the formation of. [2-nitroimidazole-IDA -$^{99m}Tc(CO)_3]^-$ (1) and [2-nitroimidazole-DETA- $^{99m}Tc(CO)_3]^+$ (2). In vivo distribution studies of the ^{99m}Tc complexes were carried out in Swiss mice bearing solid murine fibrosarcoma tumors. The radiolabeled preparation (37 MBq) were injected intravenously, in individual sets of animals (n=3). The animals were sacrificed at 0.5h, 1h and 3h p.i. and the relevant organs excised for measurement of retained activity.



1 2

Results: The 2-nitroimidazole-BFCA conjugates could be prepared in >75 % overall yield. The yields of complexation as determined by HPLC were found to be > 95 %. The $LogP_{o/w}$ values of the complexes were found to be 0.43 and 0.28 respectively. In in vivo distribution studies, while 1 showed uptake and slow clearance from tumor, 2 showed fast clearance from tumor. The retention and clearance from blood and liver also followed a similar trend. In imaging studies, unlike as in the case of 2 where the complex could not be visualized, the tumor could be visualized as early as 2 h p.i. in case of complex 1 with retention even after 24 h p.i.

Conclusions: The study revealed the influence of the resultant charge on the complex in deciding its in vivo behavior, albeit providing no clue towards the mechanism wherein the positive charge on 2 posed a negative influence on the uptake and retention in hypoxic cells. A possible explanation could be that, either entry of the complex in hypoxic cells was being prevented due to the positive charge on the complex or that the clearance of the complex from blood was fast enough to prevent reduction of the complex and accumulation in the hypoxic cells of the tumor. In vitro studies are warranted to gain insight on the exact mechanism which operates in the present case.