SYNTHESIS AND BIODISTRIBUTION OF Re/^{99m}Tc-LABELED PSMA INHIBITORS

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Introduction: Prostate Specific Membrane Antigen (PSMA) is highly expressed on the surface of prostate carcinomas. Previously, we demonstrated successful PET imaging of PSMA-expressing xenografts using the urea-based PSMA inhibitors [^{11}C]DCMC (Clin Cancer Res 2005; 11:1022), and [^{18}F]DCFBC (J Nucl Med 2006; 47:89P). Recently, we and others have shown that large substituents can be attached to an amino analog, by use of a suitable linker moiety (Mol Imaging 2006; 5:322; Chem Med Chem 2006; 1:299). Using this approach we now report the synthesis of [Re/^{99m}Tc]tricarbonyl-bisquinoline compounds **5a,b**, and -bispyridyl compounds **6a,b**, and their biodistribution in mice harboring PSMA+ and PSMA- tumors.

Experimental: Syntheses of **5a,b** and **6a,b** are shown in Figure 1. Radiochemical yields were 71% and 82%, and the radiochemical purities were 96.0% and 98.8% for **5b** and **6b** respectively. SCID mice bearing a PSMA+PC-3 Pip tumor and a PSMA- PC-3 Flu tumor on either shoulder were injected via the tail vein with 50μCi of **5b** or **6b**. Mice (4 per time point) were sacrificed at various time points. Tumor, blood, and major organs were harvested, weighed, and radioactivity was counted.

Results and Discussion: Compounds **5b** and **6b** showed specific uptake in PSMA+ tumors. At 0.5 and 1 hr PC-3 Pip tumor uptake of **5b** was 1.1±0.6 and 2.0±0.3%ID/g respectively. Compound **5b** exhibited high uptake within spleen, kidney, and small intestine. TumorT/blood (B), T/liver(L), T/spleen(Spl), T/kidney(K), T/small intest(SI), T/Large Intest(LI), and T/bladder(Bldr) ratios were 4, 0.6, 0.1, 0.02, 0.2, 1, and 2 at 0.5 hr and 6, 2, 0.1, 0.02, 1, 4, and 5 at 1 hr. Compound **6b** had PC-3 Pip tumor uptake of 7.9±4, 3.9±0.6, 2.3±0.8, and 0.8±0.5%ID/g at 0.5, 1, 2, amd 5 hr respectively. Compound **6b** exhibited rapid blood clearance with high initial Spl, K, and SI uptake. Clearance of **6b** from normal tissues was faster than from PC-3 Pip tumors resulting in increasing T/organ ratios. T/B, T/L, T/Spl, T/K, T/SI, T/LI, and T/Bldr were 15, 5, 0.7, 0.1, 1, 3.5, and 3.4 at 0.5 hr; 35, 15, 2, 0.1, 3, 0.2, and 2 at 1hr; 115, 29, 4, 0.2, 6, 2, and 0.5 at 2 hr; and 84, 21, 12, 0.7, 28, 8, and 1 at 5 hr.

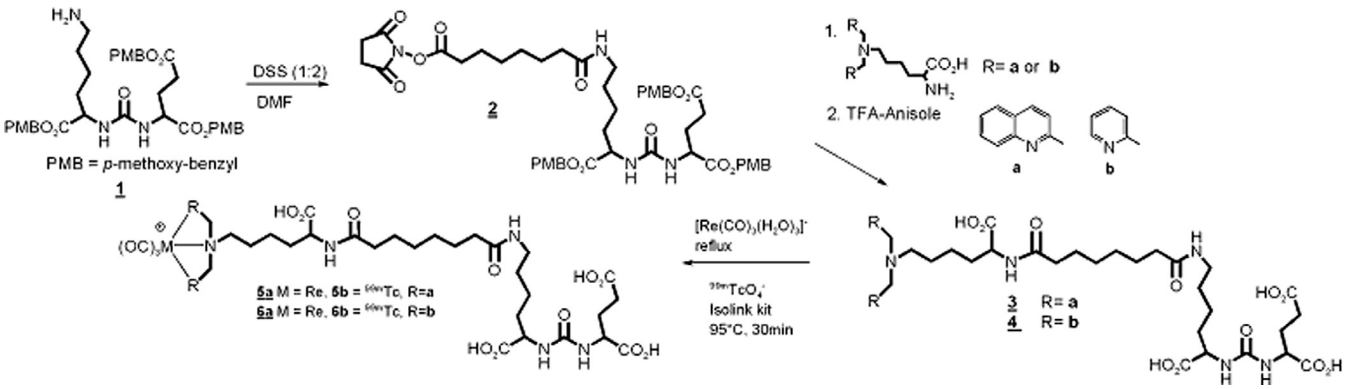

Fig. 1

Conclusion: Both **5b** and **6b** localized in PSMA+ tumor xenografts. Compound **6b** clears rapidly from most normal organs (except kidneys and bladder), resulting in high tumor/organ ratios, and is a promising candidate for SPECT imaging of Prostate Cancer.

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Keywords: Prostate Specific Membrane Antigen, Technetium Tricarbonyl, Prostate Cancer, Tumor Uptake, PSMA Inhibitor

EFFECTS OF THE ANTIFOLATE PEMETREXED ON RADIOFOLATE UPTAKE AND DISTRIBUTION IN FR-POSITIVE TUMORS AND KIDNEYS

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Introduction: The folate receptor (FR) is frequently overexpressed on diverse tumor types and can be targeted with folate-based (radio)pharmaceuticals. However, FRs are also substantially expressed in the renal proximal tubule cells resulting in high accumulation of any radiofolate. Recently, biodistribution studies in mice showed that preinjection of the antifolate pemetrexed (PMX) significantly increased tumor-to-kidney ratios of radiofolates. The goal of this study was to investigate the effects of PMX on tumoral and renal radioactivity uptake/distribution of radiofolates by *ex vivo* and *in vitro* autoradiography.

Experimental: Nude mice bearing FR-positive human KB-tumors were dissected 18 h after injection of an organometallic ^{99m}Tc-radiofolate (100 MBq) with or without PMX (400μg). Tumor and kidneys were frozen and cut into sections. Some sections were immediately applied for *ex vivo* autoradiography to study the renal and tumoral distribution of radioactivity (Fig. 1A). After decay of injected radioactivity adjacent sections were incubated with ^{99m}Tc-radiofolate for *in vitro* autoradiography to determine the FR-expression pattern throughout tumor tissue and kidneys (Fig. 1B).

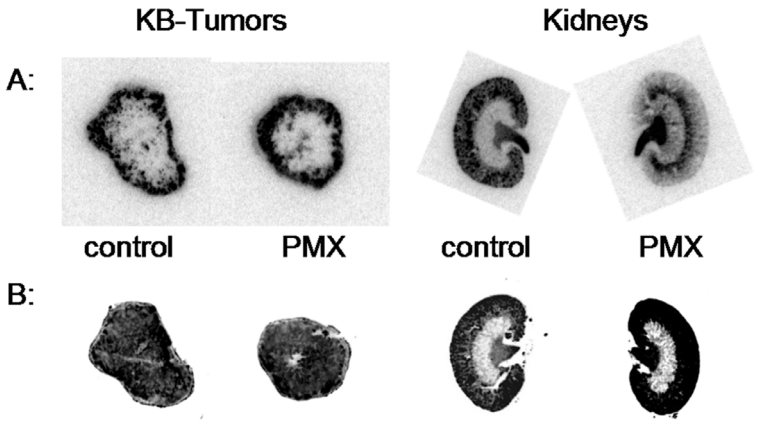

Fig. 1. A: *Ex vivo* and B: *in vitro* autoradiograms.

Results and Discussion: *Ex vivo* autoradiography after biodistribution showed accumulation of radioactivity only in the outer rim of KB-tumors in control and PMX-preinjected animals. *In vitro* autoradiograms of the tumors showed however homogenous distribution of FRs throughout the tumor tissue. *Ex vivo* autoradiograms of kidney sections from control animals showed high accumulation of radioactivity in the renal cortex. *In vitro* autoradiograms confirmed FR-expression in the renal cortex. The distribution of radioactivity in *ex vivo* autoradiograms from kidneys of PMX-preinjected animals showed significant reduction of radioactivity in the cortex indicating a FR-specific kidney blockade by PMX.

Conclusion: Our findings show that the inhibitory effect of PMX is FR-specific in kidneys but does not impair tumor uptake of the radiofolate. In nuclear medicine, we can take a profit of these effects insofar an almost 10-fold increased tumor-to-kidney ratio of radioactivity could be achieved in combination with PMX. This is of highest interest for therapeutic applications of radiofolates.

Keywords: Folate Receptor, Kidneys, Folate Radiotracer, Autoradiography, Pemetrexed

SYNTHESIS AND EVALUATION OF *N*-(2-DIETHYLAMINOETHY)-4-(^{18}F)FLUOROBENZAMIDE (DAFBA) AS PET LIGAND TO IMAGE MELANOMA

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Introduction: F-18 FDG PET imaging improved detection of melanoma but show low sensitivity. Others developed several melanoma imaging agents for SPECT imaging viz. BPB and IMBA. For PET imaging, we are developing *N*-(2-diethylaminoethyl)-4-fluorobenzamide (DAFBA). Herein, we report the synthesis, radiochemical synthesis, *in vitro* and *in vivo* properties of DAFBA.

Experimental: [^{18}F]DAFBA was synthesized in three steps. Quat. salt of benzaldehyde was reacted with TBA-[^{18}F]fluoride, followed by KMnO4 reaction and coupling to 2-diethyl aminoethylamine yielded [^{18}F]DAFBA. Binding assay was done using melanoma B16 F1 cells. For blocking, one set was incubated with 500μg of DAFBA. Binding kinetics was done at different temps. For tissue distribution, mice were injected with 20 μCi of [^{18}F]DAFBA. Mice were killed, tissues removed, weighed and counted for radioactivity.

Results and Discussion: DAFBA was prepared in ~85% yields. The [^{18}F]DAFBA was synthesized in 18 ± 5% yields and in ~180 min. Specific activity was >4 TBq/μmole. The % cell binding was 3.5 ± 1.1, 6.7 ± 0.5, and 22.6 ± 2.3 using 5x10^5, 10^6, and 5x10^6 cells, respectively. The % binding to 10^6 cells was 3.3 ± 1.9, 6.8 ± 2.2 and 11.5 ± 1.5 at 0^{0}C, 22^{0}C, and 37^{0}C. Preincubation with DAFBA significantly reduced the binding (1.2 ± 0.9%) showing a saturable process. *In vivo* studies showed an initial uptake followed by a rapid clearance of radioactivity from normal tissues with time (figure 2), with a 3-100 fold lower accumulation when compared with BPB (3). [^{18}F]DAFBA was resistant to *in vivo* deflurination (bone: 0.2 ± 0.02%ID/g).

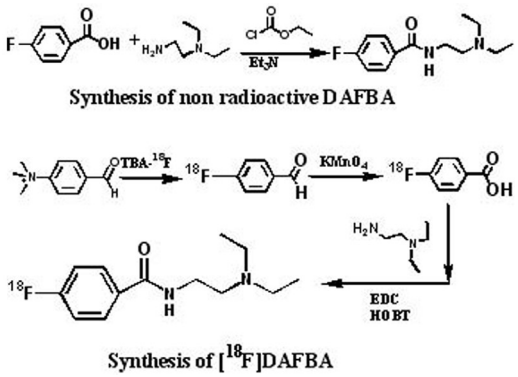

Fig. 1

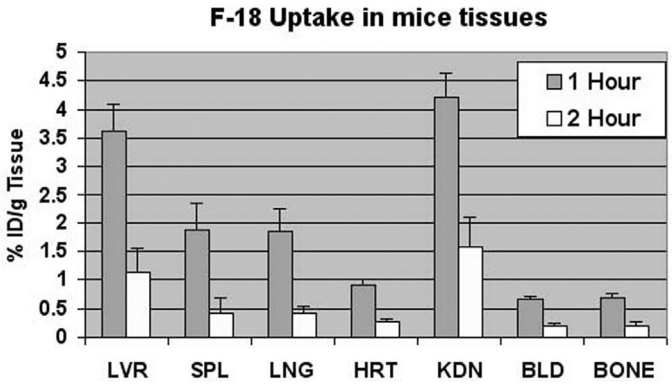

Fig. 2

Conclusion: [^{18}F]DAFBA binds to melanoma cells and show favorable tissue distribution. Furhter evaluations in tumor bearing animals and microPET studies are underway.

Acknowledgement: This project was supported in part by a grant from NIH CA105382.

Keywords: PET Imaging, Melanoma Imaging, F-18 Benzamide, Radiosynthesis, Biodistribution in Mice

CONJUGATION OF A PEGYLATED DECABORATE(2-) DERIVATIVE TO RECOMBINANT STREPTAVIDIN RESULTS IN HIGH STABILITY OF ASTATINATED PRODUCT AND IMPROVED IN VIVO CHARACTERISTICS

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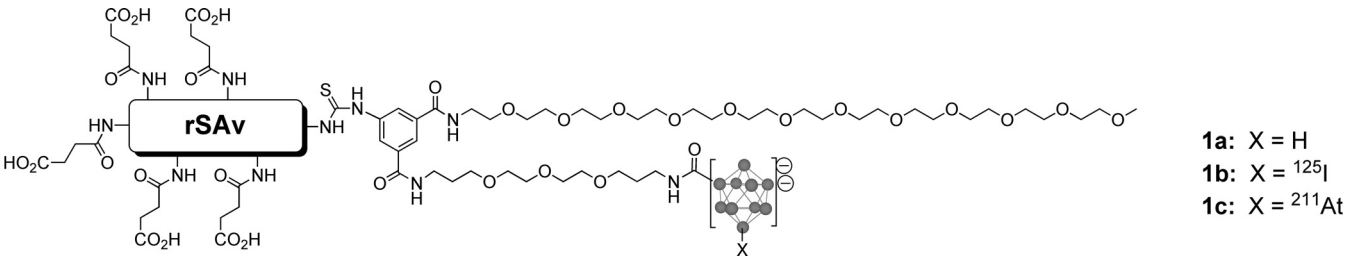
Introduction: We are investigating the use of recombinant streptavidin (rSAv) as a carrier of ^{211}At in pretargeting protocols. Although rSAv is a protein of ~52 kDa MW, previous studies have shown that succinylation keeps it from localizing in the kidneys. Other prior studies, using a *nido*-carborane group for astatination, provided a product that was stable to deastatination, but concentrations in most tissues were higher than obtained with directly astatinated rSAv. In this investigation a PEGylated decaborate(2-) derivative was evaluated as a rSAv conjugate in an attempt to obtain higher radiochemical yields and better in vivo characteristics.

Experimental: The PEG/decaborate(2-) reagent was synthesized by coupling an amino-isophthalate derivative with amino-m-dPEG™12, followed by reaction with a trioxadiamine adduct of carbonylated decaborate(2-). The resultant aniline-containing decaborate(2-) derivative was converted to a phenylisothiocyanate and was conjugated with rSAv. Following conjugation, the modified rSAv was reacted with succinic anhydride in buffer, pH 8.5, to cap all (or most) remaining surface amines. The substituted rSAv, **1a**, was radiohalogenated by reaction of the radiohalide with chloramine-T in aqueous solution to yield [^{211}At]**1c** and [^{125}I]**1b**. Biodistribution studies were conducted in athymic mice at 4 and 24 h post injection for the co-injected [^{211}At]**1c/[**^{125}**I]1b.**

Results and Discussion: Biodistribution of [^{211}At]**1c** and [^{125}I]**1b** showed no significant differences between the two nuclides in any tissue. Importantly, the blood concentration was found to be less than half (~11%ID/g @ 4h; 2%ID/g @ 24h pi) that of other rSAv conjugates studied (e.g. 21-25%ID/g @ 4h; 6-8%ID/g @ 24h pi). All other tissue concentrations were significantly lower for [^{211}At]**1c**/[^{125}I]**1b**, except for kidney and liver, which were similar to that obtained with directly labeled succinylated rSAv (no borane cage).



Conclusion: [^{211}At]**1c**/[^{125}I]**1b** have the best in vivo pharmacokinetics of the rSAv derivatives studied to date. Direct astatination was accomplished in higher yield (71%) than on succinylated rSAv (18%) or *nido*-carborane conjugated rSAv (50%). Importantly, the astatinated product was found to be very stable to deastatination. Rapid blood clearance of [^{211}At]**1c** improves its therapeutic characteristics.

Acknowledgement: We thank NIH (1R01 CA113431) for funding the studies.

Keywords: Astatine-211, Radiohalogenation, Decaborate, Streptavidin, PEG