NEW DISCOVERY STRATEGIES IN RADIOPHARMACEUTICAL CHEMISTRY

J.F. VALLIANT[1], S. PARKER[2], N. OAKLEY[1], J. ZUBIETA[3], K.A. STEPHENSON[1], L.C. REID[1], A.F. ARMSTRONG[1], G. SINGH[2] and J.W. BABICH[4]

[1]Chemistry, McMaster University, Hamilton, ON, Canada; [2]Juravinski Cancer Centre, Hamilton, ON, Canada; [3]Chemistry, Syracuse University, Syracuse, NY, USA; [4]Molecular Insight Pharmaceuticals, Cambridge, MA, USA

Introduction: The strategies used to develop molecular imaging and therapy agents have not kept pace with the technologies used in modern drug discovery programs. This is due in large part to the costs associated with high throughput discovery strategies and the lack of synthetic methods that are suited for working with radionuclides. This is particularly true in the case of radiometals where chelate complexes are not often amenable to multi-step or solid-phase synthetic protocols that are common to most drug discovery methods.

To facilitate the use of modern drug discovery techniques in Tc-radiopharmaceutical development our group created a novel series of organometalic complexes of Tc(I) and Re(I). The single amino acid chelate (SAAC) system is an amino acid analogue that can be incorporated into peptides at any position as if it were a natural amino acid. This affords the opportunity to create libraries of potential imaging agents by using a chelate scan similar to the alanine scan used in HTS.

Experimental: Using the one-bead one-compound approach, libraries of peptide-ligand derivatives that are as large as 64 million compounds were prepared by one person in under 48 hours. The Re(SAAC) complex was incorporated into a hexapeptide library for example using a mix-and-split approach with the efficiency of each coupling step monitored to ensure equimolar mixture of peptides. Protecting groups were removed using standard protocols.

Results and Discussion: By employing validated peptide synthesis methods, libraries of peptides containing the SAAC ligand were prepared. What is particularly unique about the approach is that the peptide derivatives were prepared using the Re complex of the ligand. As a result, the library constituents are exact mimics of the target Tc (and radio-Re) products so that the impact of the metal on target affinity and specificity is taken into account while screening for leads. The methodology is highly efficient and amenable to the preparation of complex peptide structures including cyclic peptides.

Conclusion: A new technique for producing libraries of peptide-based radiopharmaceuticals was developed. The methodology is designed to accelerate the process of producing novel technetium and rhenium-based molecular imaging and therapy agents.

Acknowledgement: ORDCF and NSERC of Canada for funding.

Keywords: Technetium, Peptides, Combinatorial, Discovery, Organometallic

IMPROVED PEPTIDE CHELATORS FOR Tc/Re(V); STABILIZING SIDE CHAINS

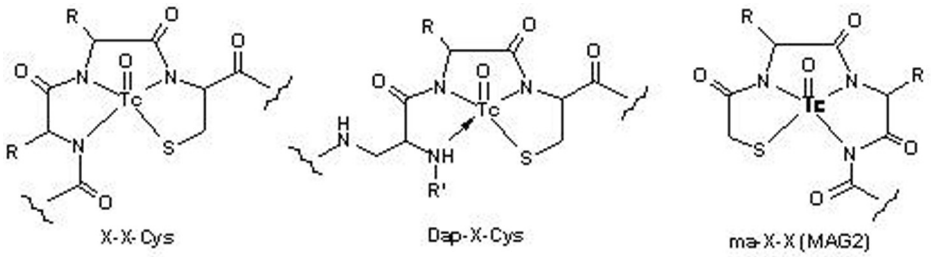
J.E. CYR, M. KOPPITZ, A. SRINIVASAN and L.M. DINKELBORG

Research Laboratories of Schering AG, Berlin, Germany

Introduction: Peptide chelators for ^{99m}Tc complex Tc/Re(V) with N_3S coordination as shown below. They offer advantages relative to traditional small-molecule chelators because they are easier to synthesize (solid phase; automated) and because they can impart greater flexibility to peptide lead-optimization/SAR programs. Several radiopharmaceuticals employ peptide chelators: for example AcuTect™ (DVT imaging), NeoTect™ (lung cancer), or EC20 (folate analog; tumor imaging).

We compared the stabilities of ^{99m}Tc somatostatin peptides with different Dap-X-Cys chelators (R' = H; R = -H, $-(CH_2)_nNH_2$; n = 1-4) and noted that those with amine side chains 3 or 4 atoms in length (i.e. X = Dab, Orn) were more stable than the others (i.e. X = Gly, Dap, or Lys). This led us to postulate that a coordinating side chain of this length ("stabilizing side chain") on the chelator ligand backbone may be capable of stabilizing Tc/Re(V) oxo complexes by helping complex the metal.

Results and Discussion: Model chelator peptides (e.g. Ac-Tyr-Gly-X-X-Cys-Ala-NH_2) bearing possible stabilizing side chains were synthesized, radiolabeled with ^{99m}Tc, and compared in cysteine challenge studies to control peptides bearing no side chain. In the X-X-Cys series, it was found that chelators with the sequences GOC, GoC, GQC, GEC, OGC, GMC, oGC, QGC, GHC, QGC, EGC, MGC, GRC were more stable than the control chelator GGC. Hence, a variety of side chains 3-4 atoms in length in either the D- or L- configuration, bearing amine, amide, imidazole, guanidinium N or thioether S donor atoms are stabilizing. Chelators with D-Orn (GoC and oGC) showed the best stability. In the Dap-X-Cys chelator series, Dap.OC, Dap.MC, and Dap.oC, and Dap(Z).GC (Z = R' = $-COCH_2CH_2NH_2$) were more stable than Dap.GC. In the ma-X-X series, ma.oC proved to be more stable than ma.GC.



Further studies with the Dap(Z).GC model chelator peptide vs. Dap.GC showed that improved stability could be translated into higher specific activity radiolabeling (i.e. less peptide needed for radiolabeling). Hence, only 1.7 nmoles (1 μg) of Dap(Z).GC model chelator peptide was needed to give a ^{99m}Tc preparation with >94% RCP for 24 h. For the Dap.GC control, none of the preparations (up to 67 nmoles) had RCP ≥90% for more than 6 h. Finally, to test the application of these chelators to a commercial product, it was observed that replacing the L-Lys in the NeoTect™ peptide chelator Dap-Lys-Cys with D-Orn, extended the shelf life (≥90% RCP) of the product from ~3 h to ~9 h.

Keywords: Technetium, Rhenium, Chelator, Peptide, Stabilized

Re-188 COMPLEXES WITH TETRADENTATE S_4 LIGANDS DERIVED FROM MESO-DMSA FOR LABELING OF BIOMOLECULES

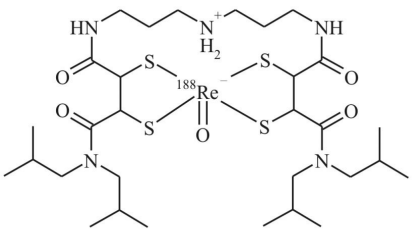
S. SEIFERT, C. JENTSCHEL, R. BERGMANN, J. STEINBACH and H.-J. PIETZSCH

Institute of Radiopharmacy, Forschungszentrum Dresden-Rossendorf, Dresden, Germany

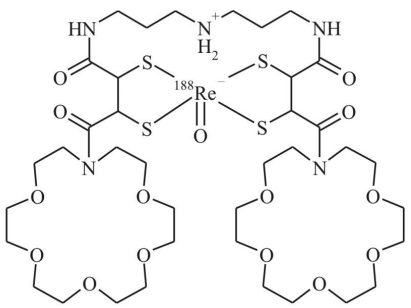
Introduction: Various Tc and Re chelates are currently under investigation for stable labeling of biomolecules. Unfortunately, most of the ^{188}Re chelates degrade more rapidly than the ^{99m}Tc analogues which is limiting the further development of rhenium-based therapeutic agents. To overcome this problem, we developed a tetradentate S_4 ligands derived from DMSA [1].

Experimental: *Labeling.* To a kit vial, containing 5 mg oxalic acid, 5 mg γ-cyclodextrin, 5 mg ascorbic acid and 0.01 - 0.05 mg Sn-S_4 complex, 1.0 ml of perrhenate eluate (50–500 MBq) was added. After heating at 50°C for 60' the yield was 90–93% (HPLC and TLC). For stability and biodistribution studies the isomers were separated by semi-preparative HPLC. The identity of the species obtained was confirmed by comparison with the HPLC profiles of fully characterized reference $^{185/187}$Re analogues. *Biodistribution.* Male Wistar rats (5–6 weeks old), 0.5 ml of the ^{188}Re complex solution (0.5 MBq) was injected into the tail vein under slight ether anesthesia. After the injection, the rats were sacrificed by heart puncture 5 min and 60 min post injection.

Results and Discussion: A kit formulation was developed which contains only microgram amounts of the S_4 ligand stabilized in the form of a stannous complex. Several isomers were separated by HPLC from the preparation solutions and characterized in vitro and in vivo. All of them were absolutely stable in rat and human plasma solutions. Challenge experiments with cysteine corroborated the high inertness of the isomers towards ligand exchange reactions. Samples of blood, intestine and urine of rats confirmed the high in vivo stability of the ^{188}Re complexes. Biodistribution studies resulted in a high uptake and fast clearance from the liver of the more lipophilic cis and trans isomers of complex **I** ($logD_{o/w}$ 1.5 - 1.7), whereas the hydrophilic isomers of complex **II** ($logD_{o/w}$ about -1.75) were preferentially excreted via the renal pathway. The low level of radioactivity in the stomach indicated good in vivo stability too.



I



II

Conclusion: The ^{188}Re-S_4 complexes offer the possibility of stable and high specific activity labeling of biomolecules. Their charge and lipophilicity may be controled by various possibilities to introduce functional groups. The amine group of the bridging framework makes available a position for coupling biomolecules.

Acknowledgement: Deutsche Forschungsgemeinschaft.

Reference: [1] S. Seifert et al., Bioconjugate Chem. 2006, 17, 1601-1606.

Keywords: Rhenium-188 Complexes, Bridged DMSA Ligand, Tetrathiol Ligand, Stability, Radiotherapy

RADIOSYNTHESIS AND PRELIMINARY EVALUATION IN RATS WITH HEPATIC INFARCTION OF A NOVEL ^{99m}Tc-LABELLED INFARCT AVID IMAGING AGENT

H.A. FONGE [1], Y. NI [2], S.K. CHITNENI [1], K. PRINSEN [1], G.M. BORMANS [1] and A.M. VERBRUGGEN [1]

[1] Lab. of Radiopharmacy, K.U. Leuven, Belgium; [2] Radiology, University Hospital (UZ) Leuven, Belgium

Introduction: The potential clinical usefulness of ^{99m}Tc-glucarate as infarct avid imaging agent is limited to the early hours of acute myocardial infarction and it is rapidly washed out from the infarcted myocardium [1,2]. In a search for a tracer agent with improved properties, we observed that derivatives of pamoic acid have a high avidity for necrotic tissue.

Experimental: We report the synthesis, radiolabelling and preliminary evaluation in normal mice and rats with hepatic infarction of the ^{99m}Tc-tricarbonyl complex of N,N'-bis(diethylenetriamino pentaacetato)-4,4'-methylene bis(1-hydroxy-2-naphthalene) carboxylic hydrazide (bis-DTPA-pamoic acid bis-hydrazide, Fig 1).

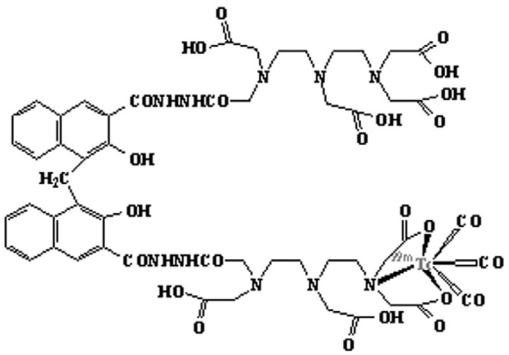


Fig. 1. Proposed structure of the ^{99m}Tc(CO)$_3$-complex with pamoic acid bis-hydrazide bis-DTPA.

Results and Discussion: The precursor was synthesized in 5 steps starting from 3-hydroxyl 2-naphthalene carboxylic acid. Radiolabelling with ^{99m}Tc(CO)$_3^+$ was achieved with a radiochemical purity of >75% using the Isolink™ kit. In normal mice, liver radioactivity was about 4% of ID/g organ from 30 min till 24 h p.i. while a significant amount was excreted via the kidneys (17.6, 25.1 and 8.6 of % of ID at 30 min, 4 h and 24 h p.i.). Blood clearance in mice was fast with 2.3% ID/g at 24 h post injection.

In rats with hepatic infarcts, the novel tracer showed high avidity for necrotic tissue with infarct:viable liver tissue activity ratios on autoradiography of 6:1 (4 h) and 8:1 (24 h). Necrosis was confirmed by histochemical staining with TTC and hematoxylin and eosin.

Conclusion: The new tracer shows high avidity for necrotic tissue and its further evaluation in different models of infarction is ongoing. The influence of a different Tc-chelator on the pamoic acid core on its necrosis avidity is being investigated and the results shall be presented.

References: [1] Ohtani H et al *J Nucl Med.* 1992;33:1988-1993. [2] Beanlands RS et al *J Nucl Cardiol.* 1997;4:274-282.

Acknowledgement: This study was funded in part by the EC - FP6-project DiMI, LSHB-CT-2005-512146.

Keywords: Bis-DTPA-Pamoate, 99mTc-Tricarbonyl, Hepatic Infarction, Necrosis Detection

SYNTHESIS, RADIOLABELING AND ANIMAL BIODISTRIBUTION OF Tc-BAT DERIVATIVES OF N-DIALKYLMINOALKYL-N-ALKYL-BENZYLAMINE FOR MELANOMA IMAGING.

A.V. JOSHUA [1], J.R. SCOTT [1], A. STRELKOV [1], A.J. McEWAN [2], S.K. SHARMA [1] and D.N. ABRAMS [1]

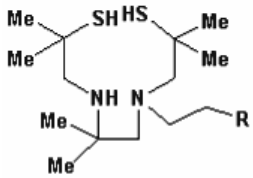
[1]Edmonton Radiopharmaceutical Centre, Alberta Cancer Board; [2]Oncologic Imaging, University of Alberta, Edmonton, Canada

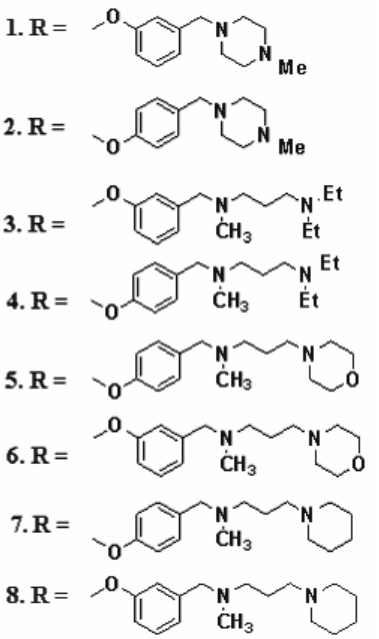
Introduction: Radioiodinated ERC-9 (N(3-(4-morpholino)propyl)-N-methyl-2-hydroxy-3-methyl-5-iodobenzylamine) which was originally developed as a pancreatic imaging agent, showed good affinity for sigma-1 and sigma-2 receptors. ERC-9 is currently being evaluated clinically for imaging of malignant melanoma and initial imaging studies with ^{123}I-ERC-9 in patients with malignant melanoma have demonstrated good uptake in the lesions.

Although ^{123}I-ERC-9 appears to have considerable potential in the localization of malignant melanoma, ^{123}I remains relatively expensive and is not routinely available. The availability of a ^{99m}Tc melanoma imaging agent, because of its almost ideal physical characteristics, availability and low cost, would have wider applicability in diagnosis and localization.

We have synthesized several compounds in which a bisaminoethanethial (BAT) moiety, which can form a neutral Tc (v) complex is linked to the basic structure of ERC-9 (N-dialkylminoalkyl-N-alkyl-benzylamine) through a three atom tether. These analogs have been labeled with Tc-99m and evaluated in animal models.

The phenolic diamines were prepared by condensing the appropriate phenolic aldehyde with the various diamines, reduction of the resulting imines with sodium borohydride in ethanol to the secondary amine followed by reductive methylation (formaldehyde, sodium cyanoborohydride in methanol). The piperazine derivatives were prepared by reductive alkylation of N-methyl piperazine with the appropriate phenolic aldehyde. Condensation of the phenolic diamines with 8-chloroacetyl-1,2-dithio-3,3,5,5,10,10-hexamethyl-5,8-diazadecane (potassium carbonate, acetone) gave the corresponding amides which reduced to the amine with aluminum hydride in THF. The disulfides were reduced to the corresponding dithiols by reduction with lithium aluminum hydride in THF.





The ligands prepared above were labelled with ^{99m}Tc ($Na^{99m}Tc04$ stannous tartrate) in 70 to 90% radiochemical yield. The neutral complexes were extracted into chloroform, the solvent removed under nitrogen and the resultant product reconstituted in 5% ethanol in saline in greater than 95% radiochemical purity. The labelled complexes were then evaluated in C57 mice bearing B-16 melanoma implants. A comparison of the biodistribution of mixed isomer preparation and the separated isomers will be discussed. In addition, the preparation and characterization of the ^{99}Tc and ^{185}Re complexes will be presented.

A NEW GALLIUM N_2S_2 COMPLEX AS A HEART IMAGING AGENT

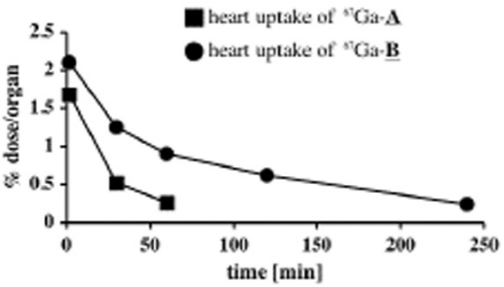
K. PLÖSSL [1], R. CHANDRA [1], B. LIEBERMAN [1], M.P. KUNG [1] and H.F. KUNG [1,2]

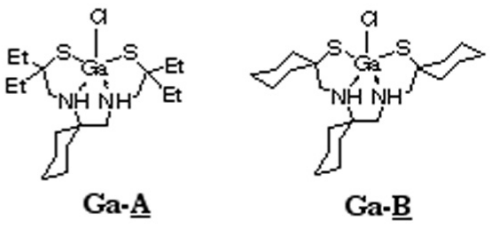
[1]Radiology, University of Pennsylvania, Philadelphia, PA, USA; [2]Pharmacology, University of Pennsylvania, Philadelphia, PA, USA

Introduction: a Previously, we have reported a neutral gallium amino thiolate complex (Ga - BAT-TECH (bis amino thiolato tetra ethyl cyclohexyl) **A (BAT-TECH)**) as a potential imaging agent for myocardial perfusion studies by PET[1,2]. The results were promising and indicated that an increase in lipophilicity - in light of the cationic nature of this compound - might improve the heart retention.

Experimental: We synthesized a new bis amino bis thiolate ligand **B** containing additional cyclohexyl rings and prepared the corresponding gallium complex. The new complex with three cyclohexyl groups - formally two more CH_2 groups compared to **A** - should be more lipophilic.

A lyophilized sample kit (0.5 mg ligand (**B** HCl salt); 1 μmol) was dissolved in 400 μL water (pH of this solution was 6) and 300 μL Ga-67-citrate solution (495 μCi) was added (pH 6). The sample was heated in a heating block at 80°C for 60 minutes. The reaction mixture was cooled to room temperature and a small sample was used for RCP determination (TLC: silicagel, acetone/acetic acid 3/1). ($R_{f\,(Ga67\text{-}B)}$ = 0.8; $R_{f\,(Ga67\text{-}citrate)}$ = 0.13; RCP = 92%).





Comparison of heart uptakes of ^{67}Ga-**B** and ^{67}Ga-**A** in rats (% dose/organ).

Results and Discussion: *In vivo* biodistribution of this novel ^{67}Ga-ligand was carried out in normal rats and it exhibited excellent heart uptake and retention with a heart uptake of 2.1% dose/organ compared to 1.68% dose/organ (Ga-**A**) after 2 minutes and a retention of 0.9% dose/organ, compared to 0.26% dose/organ for Ga-**A** after 60 minutes.

Conclusion: In conclusion, the new ligand **B** can be labeled similarly as observed for the BAT-TECH (**A**) ligand in good yield with RCP > 90%. Biodistribution in rats revealed a higher initial heart uptake and a higher retention compared to the original Ga-BAT-TECH. Further studies of the cold gallium chemistry and labeling with ^{68}Ga are currently on the way.

References: [1] Francesconi, L.C.; Liu, B.-L.; Billings, J.J.; Carroll, P.J.; Graczyk, G.; Kung, H.F. Journal of Chemical Society, Chemical Communications 1991, 2, 94-95. [2] Kung, H.F.; Liu, B.-L.; Mankoff, D.; Kung, M.-P.; Billings, J.J.; Francesconi, L.C.; Alavi, A. Journal of Nuclear Medicine 1990, 31, 1635-1640.

Keywords: PET Imaging, Myocardial Perfusion, Ga67/68 Labeling, *In Vivo* Biodistribution

^{68}Ga-LABELLING OF A FREE AND MACROMOLECULE CONJUGATED MACROCYCLIC CHELATOR AT ROOM TEMPERATURE

I. VELIKYAN [1], Y. ANDERSSON [1] and B. LANGSTROM [1,2]

[1]Uppsala Imanet AB, GE Healthcare, Uppsala, Sweden; [2]Department of Biochemistry and Organic Chemistry, BMC, Uppsala University, Uppsala, Sweden

Introduction: A straightforward labelling using generator produced positron emitting ^{68}Ga, which provids high quality images, may result in kit type production of PET radiopharmaceuticals and make PET examinations possible also at centres lacking accelerators. The introduction of macrocyclic bifunctional chelators that would provide fast ^{68}Ga-complexation at room temperature would simplify even further tracer preparation, allow for "shake and shoot" type production and open wide possibilities for ^{68}Ga-labelling of fragile and potent macromolecules. ^{68}Ga has the potential to facilitate development of clinically practical PET and to promote PET technique for individualized medicine.

Experimental: Complexation with^{68}Ga and ^{69,71}Ga was performed at room temperature, in buffer solutions. TLC, HPLC and MS were used for analysis. Stability of ^{68}Ga-NOTA complex was monitored by TLC.

Results and Discussion: Macrocyclic chelator, 1,4,7-triazacyclononanetriacetic acid (NOTA), and its derivative coupled to an eight amino acid residue peptide (NODAGATATE) were labelled with ^{68}Ge/^{68}Ga-generator produced positron emitting ^{68}Ga. Formation kinetics of ^{68}Ga-NOTA was studied as a function of pH values and formation kinetics of ^{68}Ga-NODAGATATE was studied as a function of the bioconjugate concentration. The quantitative radioactivity incorporation (RAI>95%) for ^{68}Ga-NOTA was achieved within less than 10 min at room temperature and pH 3.5. The amount of NODAGATATE required for RAI of >90% and >95% was respectively 2-5 and 10 nanomols. In both cases the purification of the ^{68}Ga-labelled products was not necessary since the radiochemical purity was >95% and the preparation buffer, 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) is eligible for human use. In order to confirm the identity of the products, complexes comprising gallium stable isotope were synthesized and analyzed by mass spectrometry. The complex was found to be stable in the reaction mixture, phosphate buffer and human plasma during 3 hour incubation.

Conclusion: Free and peptide conjugated NOTA formed stable complex with ^{68}Ga at room temperature within 10 minutes. This might be of special interest for the labelling of fragile and potent macromolecules and allow for "shake and shoot" type preparation of ^{68}Ga-based radiopharmaceuticals.

Acknowledgement: This work was partly sponsored by Medical Diagnostics, GE Healthcare and we acknowledge Peter Iveson and his colleagues for the supply of NOTA. We sincerely acknowledge Professor Helmut Maecke, Institute of Nuclear Medicine, Basel, Switzerland, for the provision of NODAGATATE.

Keywords: 68Ga, NOTA, PET, Kit Production, Bioconjugate