Radiopharmaceutical Development

Introduction

Radiopharmaceuticals, drugs containing a radionuclide, are a fundamental and essential component in Nuclear Medicine studies directed toward diagnosis or treatment of disease in patients. The aim of the project is to promote drugs-oriented research essential focused on the improvement or development of new ^{99m}Tc and ^{123}I/^{125}I/^{131}I radiopharmaceuticals of suitable quality for clinical diagnostic purpose.
The most relevant results obtained in this project are outlined:

- ^{99m}Tc-compounds (human polyclonal immunoglobulin (HIG) and ciprofloxacin (CIP)) as inflammation imaging agents were investigated. HIG was labelled with ^{99m}Tc by a novel MAG_3 labelling method and CIP as proposed by Vinjamuri. Both radiopharmaceuticals were chemical, radiochemical and biologically characterized and their value for imaging of bacterial *versus* sterile inflammations compared. Neither HIG nor CIP distinguished bacterial from sterile inflammations. Nevertheless, with higher inflammation uptake, labelled HIG may be considered superior as agent for inflammation imaging.

- Synthesis and characterization of complexes of ^{99m}Tc with mono thiol peptide derivatives and a tridentate dithiol ligand. Biological studies will be performed and correlated with their radiochemical characteristics.

- Dinamic gamma-camera studies of the neutral complex ^{99m}Tc-Big (Big=Biguanide) in rats and rabbits, have shown that it could be a potential ^{99m}Tc-DMSA replacement renal imaging agent. In order to evaluate ^{99m}Tc-Big in baboons, before humans clinical trials, experiments leading to a formulation kit were developed.

- Molecular generated structures of some Tc-biguanide complexes are predicted by molecular mechanics calculations and their interactions simulations with water molecules or peptide chains, are correlated with experimental data of partition coefficients and percentage of human protein binding. The results stress the interest of molecular modeling to predict molecular properties.

- The synthesis and radiochemical characterization of oxo cationic complexes of technetium-99m with biguanide and the N-1 derivative dimethyl and phenethyl, were developed.

- Synthesis and characterization of two new estradiol derivatives: (17α,20*E*)-and (17α,20*Z*)-[^{125}I]Iodovinyl-3-methoxy-6-dehydroestradiol. The potential value of these new compounds as estrogen receptor imaging agents will be evaluated by studying their biological properties.

- Investigation on the pharmacological profile of the receptor mediating the inhibitory action of NPY on the gonadotropic axis by using a variety of NPY analogs with different selectivity towards the 5 NPY receptors subtypes suggested that the receptor involved in negative control by NPY of the gonadotropic axis is distint from the Y1-Y4 subtypes but bears many similarities with the pharmacology of the Y5.

Research Team

Researchers	6	(2 PhD or equivalent)
Technicians	3*	

* 1 with grant from FCT

Publications

Journals	3	(2 in press)
Conf. Commun. –	8	

10^3 PTE

Expenditure:	**20.076**
Missions:	1.064
Others Expenses:	5.731
Hardware & Software:	3.643
Other Equipment:	9.638

10^3 PTE

Funding:		**23.386**
External Projects:	1996	5.040 [(1)]
	1997	17.075
Others		271

[(1)] Funding not used in 1996

^{99m}Tc-RADIOPHARMACEUTICAL DEVELOPMENT

Human Polyclonal Immunoglobulin labelled with Technetium-99m Via NHS-MAG_3: a Comparison of Radiochemical Behavior and Biological Efficacy with other Labelling Methods

L. Gano[1], L. Patrício[1], E. Marques[2], G. Cantinho[3], H. Pena[3], T. Martins[3], D. J. Hnatowich[4]
[1]*Instituto Tecnológico e Nuclear, 2686 Sacavém codex, Portugal*
[2]*Instituto Nacional de Engenharia e Tecnologia Industrial, IBQTA/Lab. de Microbiologia Industrial, 1600 Lisboa, Portugal*
[3]*Instituto Medicina Nuclear, F.M.L., 1600 Lisboa, Portugal*
[4]*University of Massachusetts Medical Center, Worceste, MA, USA.*

Abstract
The aim of this study was to evaluate the radiochemical behavior, biological distribution and localization in infection sites in mice of a human polyclonal immunoglobulin (HIG) labelled with ^{99m}Tc by a novel MAG_3 labelling method. The resulting [^{99m}Tc]MAG_3-HIG was compared with [^{99m}Tc]HIG preparations radiolabelled directly via 2-mercaptoethanol (2-Me) or stannous ion (Sn) reduction and indirectly via 2-iminothiolane (2-Im) conjugation. All preparations showed similar UV and radioactivity HPLC profile to that of native HIG except for 2-Im-HIG which showed aggregates. The stabilities of the label to challenge with cysteine were similar for all the preparations. By non-denaturing SDS PAGE, all preparations other than MAG_3-HIG showed evidence of lower molecular weight fragments. The tissue distribution 4 and 24 h after intravenous administration of the four preparations were compared in healthy mice and in mice previously administered with an isolate of *S. aureus* in one thigh. The pharmacokinetics varied among the different preparations. When prepared via 2-Me, Sn and 2-Im, blood clearance and urinary excretion were faster than that of labelled MAG_3-HIG. The absolute uptake in the infected thigh at 24 h was significantly higher for HIG labelled via MAG_3 and 2-Me vs. the remaining methods. The infected thigh/normal thigh radioactivity ratios were similar at both time points for labelled HIG prepared via 2-Me, 2-Im and NHS-MAG_3 methods but was significantly lower at 24 h for HIG prepared via Sn. The radioactive HPLC profiles of serum, at 4 and 24 h were similar to that of the radiolabelled injectates. Based on these data we conclude that each radiolabelled HIG preparation studied showed increased localization in infectious foci although [^{99m}Tc]MAG_3-HIG showed superior radiochemical and biological characteristics under the conditions of this investigation.

Nuc. Med. Biol.(in press).

A Molecular Mechanics Based Study of Biguanide Tc-99m Complexes and their Interactions with Water, Peptides and Phospholipids

M. Neves[1], A. Marchi[2] , V. Bertolasi[2] and R. Fausto[3]
[1]*Instituto Tecnológico e Nuclear, Sacavém , Portugal*
[2]*Dipartimento di Chimica, Università di Ferrara, Ferrara, Italy*
[3]*Departamento de Química, Universidade de Coimbra, Portugal*

Abstract
Prediction of the structure of Tc-99m complexes by computer-aided methods has been rarely used. However, since most of the time it is not possible to get crystals of the Tc-99 complexes suitable for complete structural caracterization by X-ray crystallography, computational chemistry methods may constitute a very powerful and convenient alternative to the experimental techniques, in particular when the theoretical predictions are complemented with results obtained by elemental analyses and spectroscopic techniques. In this study, a series of biguanide Tc complexes have been studied by molecular mechanics, using the PCMODEL MMX-pi force field, that performs pi-VESCF calculations in addition to the conventional σ-system molecular mechanics calculations. The reliability and accuracy of this technique to study such systems was confirmed by a detailed comparison between the theoretically predicted geometrical parameters (atomic distances, valence and dihedral angles) obtained for the cationic complexes $[TcO(DMBig)_2]^+$ and $[TcN(DMBig)_2\ (OH_2)]^{2+}$ (DMBig=dimethyl-biguanide) and the available experimental X-ray crystalographic data. In addition to the simpler structural predictions, the computational method used in this study also enable a detailed evaluation of the main intermolecular interactions between the studied Tc- complexes and molecules as water, peptide chains and simple phospholipids, as well as to estimate the relative lipophilicity of the various studied species. Finally, a correlation between both interaction energies and surfaces areas of the low-energy conformations and results of *in vitro* and *in vivo* studies was established.
*Acknowledgements:*This work is finnancially supported by the PRAXIS XXI reseach projects QUI/2/2.1/412/94, SAU/2/2.1/1396/95 and FEDER.

Communication to: *10th International Symposium in Radiopharmacology*, Rapallo, Génova, Itália, 17-23 Maio 1997. Published in *The Quartely Journal of Nuclear Medicine*, **41**, Supl.1 to n.2, 1997.

---

^{99m}Tc-Human Polyclonal Immunoglobulin Via NHS-MAG_3: Comparison of Radiochemical Behavior and Biological Efficacy with other ^{99m}Tc-Immunoglobulin

L. Gano[1], L. Patrício[1], A. Rodrigues[1] , E. Marques[2], D. Hnatowich[3]
[1]*Instituto Tecnológico e Nuclear, Sacavém; Portugal*
[2]*Instituto Nacional de Engenharia e Tecnologia Industrial, IBQTA/ Lab. de Microbiologia Industrial, Portugal*
[3]*University of Massachusetts Medical Center, Worcester, MA, USA*

Abstract
Radiolabelled human non-specific polyclonal immunoglobulin (HIG) have been proposed for the scintigraphic detection of focal sites of infection/ inflammation. Recently Hnatowich et al.

introduced a new method for ^{99m}Tc radiolabelling of biomolecules by conjugation with a bifuntional chelate, an acetyl protected esther of MAG_3, NHS-MAG_3. Based on this chelator an indirect method for labelling of HIG was improved. The radiochemical properties, biological distribution and binding affinity to infection sites in mice of the new ^{99m}Tc-HIG preparation were compared to ^{99m}Tc-HIG prepared through well established methods: 2-mercaptoethanol (2-Me) reduction, stannous ($SnCl_2$) redution and 2-iminothiolane (2-Im) coupling.
Labelling efficiency was higher than 98% for ^{99m}Tc-HIG preparations obtained by 2-Me redution and 2-Im coupling. Stannous reduction (labelling yield of 60-85%) conjugation with NHS-MAG_3 (labelling yield 70-90 %) implied a purification step post-labelling. The HPLC analysis of the labelled preparations indicate that all of them contained some aggregates or polymeric fractions however 2-Im coupling induces the highest polymeric contamination. SDS PAGE analysis under non reducing conditions revealed the presence of other protein fractions of lower molecular wheight than intact IgG on labelled HIG preparations. The reducing agents as well as 2-Im significantly modify the profile of the native HIG and present significant high radioactivity associated to the low MW fractions. NHS-MAG_3 conjugated HIG presented about a quite similar pattern to that of native HIG.
The *in vivo* behavior of the ^{99m}Tc -HIG preparations was evaluated in normal female mice. Animals were intravenously injected and were killed 4 h and 24 h post-injection. Different biodistribution profiles were found, especially in blood clearance and urinary excretion which were significantly higher ($p<0.05$) with HIG modified by 2-Im and lower in the case of HIG conjugated with NHS-MAG_3 ($p<0.01$). The binding affinity to infection sites of the four ^{99m}Tc-HIG preparations was studied in mice with thigh abcess induced by im. administration of 2 x 10^8 colonies of *S. aureus* at 4 and 24 hours after ^{99m}Tc -HIG injection. Results were expressed as the count ratio for infected / non infected thigh. At 4 h the ratios obtained were 3.8 ± 0.8, 3.8 ± 0.7, 4.2 ± 1.0 and 4.6 ± 0.9 respectively for 2-Me, $SnCl_2$, 2-Im and NHS-MAG_3 preparations. At 24 h, the ratios were 7.3 ± 1.2, 4.0 ± 0.6, 6.0 ± 0.8 and 7.0 ± 1.2 respectively. In conclusion, at 24 h the ^{99m}Tc uptake in the abcess was significantly lower for HIG ($SnCl_2$). For the other HIG preparations no significative diferences were found ($p<0.05$). Although labelling efficiencies were lower, the radiochemical purity of ^{99m}Tc-HIG appeared to be superior when prepared via NHS-MAG_3.

Communication to : *7th European Symposium on Radiopharmacy and Radiopharmaceuticals*, 2-5 March 1997.

---

^{99m}Tc-Immunoglobulin *versus* ^{99m}Tc-Ciprofloxacin: Comparative Study in Infected mice

L. Patrício[1], L.Gano[1], E. Marques[2], Guilhermina Cantinho[3]
[1]*Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2685 Sacavém*
[2]*Instituto Nacional de Engenharia e Tecnologia Industrial, IBQTA/ Lab. de Microbiologia Industrial, 1600 Lisboa, Portugal*
[3]*Instituto Medicina Nuclear, F.M.L., 1600 Lisboa, Portugal*

Abstract
The aim of the study was to compare the value of ^{99m}Tc-Immunoglobulin (^{99m}Tc-HIG) and ^{99m}Tc Ciprofloxacin (^{99m}Tc-CIP) as infection imaging agent. Two different formulations of ^{99m}Tc-HIG were used: prepared via 2- mercaptoethanol (2-Me-HIG) reduction and NHS-MAG_3 conjugation (MAG_3-HIG). Both preparations 2-Me-HIG and MAG_3-HIG were

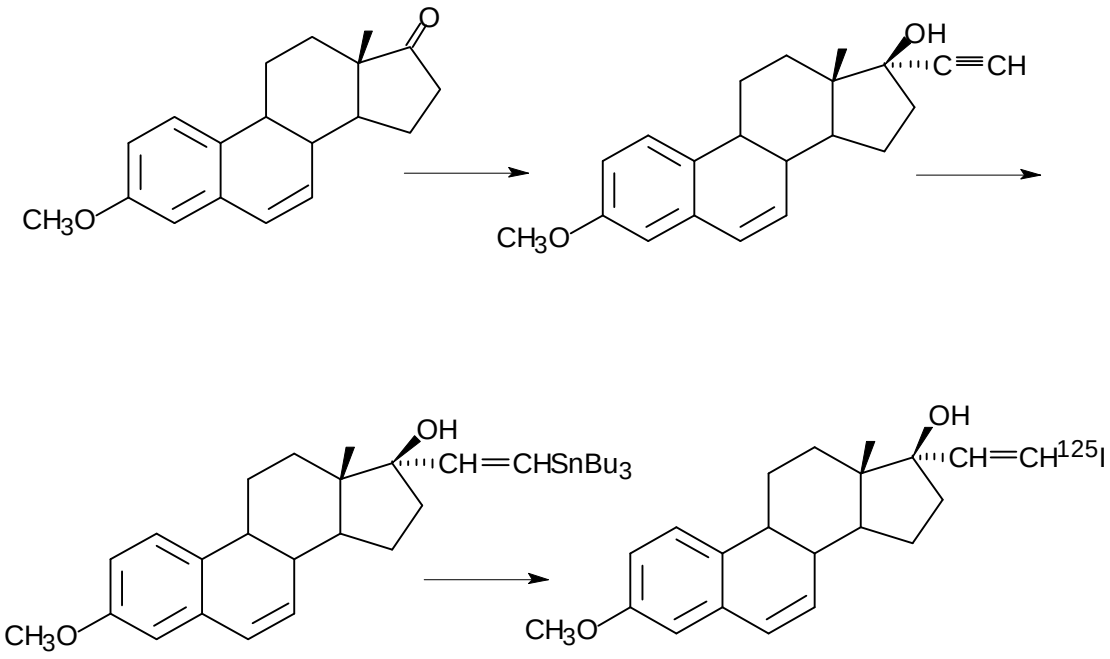
chemical and radiochemically characterized by ITLC, HPLC and SDS PAGE and the biokinetics evaluated in mice. Radiolabelling of CIP was accomplished according Vinjamuri with a labelling efficiency higher 95%. Therefore no post-labelling purification was required. Biological activity studies performed with CIP in presence of FSA and heated at 100º C and CIP (starting material) by using *S. aureus* and *E. coli* cultures, made evidence that CIP did not loose activity under chemical conditions for labelling purpose. The biokinetics of the radiopharmaceuticals was studied in infected mice. The tissue uptake express in % of injected dose, 4 h after administration was determined. The binding affinity to infection sites of the preparations under study was studied in mice with thigh abscess induced by i.m. administration of *S. aureus* and *E. coli* (~$2x10^7$ colonies). Results were expressed as the count ratio target thigh/ normal thigh. The target thigh/ normal thigh ratios for HIG preparations (3.8 ± 0.8 for 2-Me-HIG and 4.6 ± 0.9 for MAG_3-HIG) were statistically superior than that observed for CIP 2.1 ± 0.09.

Communication to: *XIX Congresso Nacional de Medicina Nuclear,* Alicante, Espanha, 12-14 Junho 1997.

17α-[^{125}I]iodovinyl derivatives of 3-methoxy-6-dehydro estradiol: potential radioligands for the estrogen receptor

M. C. Melo e Silva[1], T. Thiemann[3], L. Patrício[1], M. L. Sá e Melo[2]
[1]Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2685 Sacavém
[2]Centro de Estudos Farmacêuticos, L. Q. F., Fac. Farmácia, Univ. Coimbra, 3000 Coimbra
[3]Faculté des Sciences Appliqueés,ULB, B-1050 Bruxelles

Abstract
As part of our program for the development of new radiolabeled estrogen derivatives as potential receptor imaging agents for human breast tumors, we undertook the synthesis and characterization of (17α,20E)-and (17α,20Z)-[^{125}I]Iodovinyl-3-methoxy-6-dehydroestradiol starting from 17α- ethynyl derivative previously prepared[1]. Stereoselective formation of the E and Z vinylstannyl isomers from 17α- ethynyl precursor was controlled by the presence or absence of catalyst, the polarity of solvent and the reaction temperature.[2] Stereochemical assignments are based on ^{1}H NMR and ^{13}C NMR spectroscopy. The 17α-iodovinyl estradiol derivatives were prepared with retention of configuration, by destannylation of the corresponding tributylstannyl intermediates. The radiolabelled [^{125}I] analogues were obtained in good radiochemical yield and high purity by treatment of the tributylstannyl precursors with [^{125}I]NaI, in the presence of chloroamine-T, and purification by HPLC. The potential value of these new radiopharmaceuticals as estrogen receptor imaging agents will be evaluated by studying their biological properties.



References:
1. Tedesco, R., Fiaschi, R., Napolitano, E., (1995) *Synthesis*, 1493
2. Ali, H., Rousseau, J., Ghaffari, M. A., van Lier, J.E. (1991) *J. Chem. Soc. Perkin Trans.*1, 2485

♣ This research is financially supported by PRAXIS XXI, research project PSAU C/SAU/89/96 and FEDER.

Communication to: *2º Encontro de Química Orgânica*, Oeiras, Portugal, 17-19 Setembro 1997.

Stereoisomers of 17α-[^{125}I]iodovinyl-6-desidrostradiol-3-methyl Eter: Potential Ligands as Estrogen Receptor Imaging

M. C. Melo e Silva[1], T. Thiemann[3], L. Patrício[1], M. L. Sá e Melo[2]
[1]*Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2685 Sacavém*
[2]*Centro de Estudos Farmacêuticos, L. Q. F., Fac. Farmácia, Univ. Coimbra, 3000 Coimbra*
[3]*Faculté des Sciences Appliqueés,ULB, B-1050 Bruxelles*

Abstract
Radioiodinated estrogen derivatives have been proposed as agents for breast cancer imaging[1]. In the present study we report the synthesis and characterization of (17α,20*E*)- and (17α,20*Z*)-17α-[^{125}I]iodovinyl-6-dehydroestradiol-3-methyl ether. The stereoselective formation of *E* and *Z* vinyilstannyl isomers prepared from the 17α-ethynyl derivatives was controlled by the presence or absence of catalyst, the polarity of the solvent and the reaction temperature[2]. Their stereochemical characterization was accomplished by ^{1}H and ^{13}C NMR spectroscopy. The stereoisomers of 17α-iodovinyl-6-estradiol were prepared with retention of configuration from the correspondent tributylstannyl intermediates. The 17α-[^{125}I]iodovinylestradiol analogues

were obtained from the tributylstannyl precursors with [^{125}I]NaI in presence of chloroamine-T and subsequent HPLC purification. The potential value of these new estradiol derivatives synthesized will be evaluated by studying their biological properties.

References:
1. Riks, L. J. M., Boer G. J., Endert E. et al. (1997) Nuc. Med.Biol. **24**: 65
2. Ali, H., Rousseau, J., Ghaffari, M. A., van Lier, J.E. (1991) *J. Chem. Soc. Perkin Trans.*1, 2485

♣ This research is financially supported by PRAXIS XXI, research project PSAU/C SAU/89/96 and FEDER.

Communication to: *V Curso de Divulgação de Medicina Nuclear/Avanços Recentes em Oncologia,* Lisboa, Portugal, 24-25 de Outubro 1997.

Evidence for a Leptin-Neuropeptide Y Axis for the Regulation of Growth Hormone Secretion in the Rat

[1]Dominique D. Pierroz, [1]Beatrice A.M. Vuagnat, [2]Paula D. Raposinho, [1]François P. Pralong, [1]Werner F. Blum and [1]Michel Aubert
[1]Dept of Pediatrics, Univ. of Geneva School of Medicine, 1211 Geneva 14; Dept of Medicine, Univ of Lausanne, 1011 Lausanne, Switzerland, and University of Giessen and Lilly Deutschland, Bad Homburg, Germany.
[2]Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2685 Sacavém, Portugal

Abstract
Hypothalamic gene expression for Neuropeptide Y (NPY) is augmented in adverse metabolic conditions such as food restriction. We have shown that central infusion of NPY, that mimics the presence of elevated NPY, fully inhibits Growth Hormone (GH) secretion, and that a bolus NPY injection acutely inhibits GH secretion for 3-5h pointing to a physiological role of NPY in the regulation of GH secretion. Leptin has been shown to exert control on metabolic processes at the level of the central nervous systems, in particular on food intake, and also to modulate pituitary hormone secretions in function of metabolic conditions, at least partly through a NPY neurotransmission. In the rat, GH secretion is dependent upon prevailing metabolic conditions. We reasoned that leptin could also exert control over GH secretion and we tested this hypothesis by using a three-day fast model in the male rat. Circulating leptin concentration fell to low values, <5% of control, after 24 h fasting and remained low thereafter. Upon refeeding, leptin secretion regularly increased. Plasma IGF-I also markedly decreased during fasting but according to a different pattern. As shown by others, pulsatile GH secretion disappeared after 3-day of fasting. In order to investigate whether the inhibition of GH secretion seen during fasting is related to the very low plasma leptin concentration, fasting rats were infused centrally with mouse leptin (10 mg/d, icv, Lilly laboratories). This infusion of leptin totally prevented the disappearance of GH secretion, maintaining a normal pulsatile pattern of secretion. Infusing the same amount of leptin icv to fed rats produced a severe reduction in food intake (33.9% of intake by controls) but no changes in GH secretory pattern, whereas pair-fed rats exhibited a marked reduction in GH secretion. Gene expression for NPY, estimated by Northerm blot analysis, was significantly increased in fasting rats, and reduced in leptin-treated, fasting rats. In

fed rats, leptin treatment that produced a severe reduction in food intake, also reduced gene expression for NPY, whereas pair-fed animals logically exhibited increased gene expression. In both situations with reduced feeding, normal GH secretion was seen in leptin-treated animals exhibiting low NPY gene expression, and decreased or abolished GH secretion, in animals not receiving leptin and exhibiting increased NPY mRNA density. In summary, the regulation of GH, at least the changes linked with altered metabolic conditions seen in fasting, appears to be dependent upon leptin, and probably circulating levels of this new hormone. Although a direct demonstration is still lacking, the presented data are consistent with a central effect of leptin maintaining normal pulsatile GH secretion by preventing the documented inhibitory action of NPY on GH secretion.

Communication to: 4th International NPY Conference 1997, Londres, England, 13-14th November 1997.

Synthesis, Characterization and Biodistribution of Oxo Complexes of Technetium-99m with Biguanide and N1 Substituted Ligands

M. Neves[1], L. Gano[1], M. João Ribeiro[2], A. Cristina Santos[2], A. Marchi[3] and J. J. Pedroso de Lima[2]

[1]Departamento de Química, Instituto Tecnológico e Nuclear, 2685-Sacavém, Portugal

[2]IBILI, Serviço de Biofisica, 3000-Coimbra, Portugal

[3]Dipartimento di Chimica, Università di Ferrara, 44100-Ferrara ,Italia

Abstract

We report the synthesis, characterization and biodistribution of ^{99m}Tc- -complexes with the bidentate-N,N chelate biguanide (Big) and the N1 substituted ligands DMBig, PBig and PEBig. Dynamic gamma-camera studies with ^{99m}Tc-Big and ^{99m}Tc-DMSA in rabbits indicate distinct renal and urinary excretion profiles. ^{99m}Tc-Big is cleared more quickly than ^{99m}Tc-DMSA, and for the same acquisition times, the contrast in whole-body images favoured ^{99m}Tc-Big. Also, the estimated radiation absorbed doses by kidneys and blood for ^{99m}Tc-DMSA are significantly higher than for ^{99m}Tc-Big. These preliminary studies show that ^{99m}Tc-Big has favourable practical and dosimetric features for renal imaging as an alternative to ^{99m}Tc-DMSA.

Nucl. Med. Biol.(submitted).

Prediction of ^{99m}Tc-Biguanide Complex Structures and their Interactions with Biological Molecules by Molecular Mechanics Calculations

M. Neves[1] and R. Fausto[2]
[1]*Departamento de Química, Instituto Tecnológico e Nuclear, p-2685 Sacavém, Portugal*
[2]*Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, p-3049 Coimbra, Portugal*

Abstract
The structures of some Tc-biguanide complexes are predicted by molecular mechanics calculations. In addition, simulations of molecular interactions between the predicted equilibrium structures with water molecules or peptide chains, are correlated with experimental data of partition coefficients and percentage of human protein binding, evaluated for the analogous ^{99m}Tc-biguanide complexes. These results suggest the value of computer assisted design of new Tc-radiopharmaceuticals, and in particulary, stress the great interest of using molecular modelling to predict molecular properties which can be sucessfully correlated with results obtained by *in vitro* studies.

Nucl. Med. Biol. (submitted).

Ciprofloxacin in Imaging of Infective *versus* Sterile Inflammation

L. Gano[1], L. Patrício[1], G. Cantinho[2], H. Pena[2], T. Martins[2] and E. Marques[3]
[1]*Instituto Tecnológico e Nuclear (ITN), 2686 Sacavém Codex, Portugal,*
[2]*Instituto Medicina Nuclear, F.M.L., 1600 Lisboa, Portugal*
[3]*Instituto Nacional de Engenharia e Tecnologia Industrial (INETI), 1600 Lisboa, Portugal*

Abstract
Ciprofloxacin (CIP) radiolabelled with ^{99m}Tc was proposed as a specific agent for differential diagnosis between infectious and non infectious inflammations [1]. The aim of the present work was to determine the *in vitro* and *in vivo* bacteria binding of ^{99m}Tc ciprofloxacin and to verify whether ^{99m}Tc ciprofloxacin also binds to sterile abscess *in vivo.* CIP was labelled with ^{99m}Tc by Vinjamuri method. The labelling efficiency, radiochemical purity and stability of the ^{99m}Tc-CIP were accomplished by: a) ITLC SG with saline as eluent. The labelled CIP and colloids remain at the origin while pertechnetate migrate (R_f =1). The non labelled ciprofloxacin, detected by UV, migrates with Rf = 0.5 - 0.6. b) Sephadex G-50 column (0.9×15 cm) using as eluent 0.9% sodium chloride, pH =2.5. ^{99m}Tc-CIP is eluted while the colloids remain on the top of the column. Radiolabelled efficiency of ^{99m}Tc-CIP was approximately 95%. The biological activity of CIP submitted to chemical reactions for labelling and that of CIP (drug) was evaluated by a microbiological assay. The test microorganisms were *S. aureus* and *E. coli.* The results indicated that no lost of biological activity of the CIP occurred during the radiolabelled procedure.
The *in vitro* and *in vivo* bacteria binding of ^{99m}Tc-CIP was studied using also *S. aureus* (SA) and *E.Coli* (EC). For i*n vitro* binding determination aliquots of labelled CIP were added to bacterial suspension (~10^8 bacterias). The bacterial suspensions were then incubated,

centrifuged and the radioactivity of pellets measured. The *in vitro* binding was express as percent of total activity per pellet. The ^{99m}Tc-CIP binding to SA and EC were 2.2 and 1.4%.
For *in vivo* binding evaluation SA and EC infections were experimentally induced in mice. Sterile inflammations were also induced by i. m. injection of 100 μL of turpentine oil (TURP). Twenty four h later the labelled CIP was intravenously administered. At 4 and 24 h a. a. the animals were sacrificed. Tissue samples were then removed for counting. The results were expressed as percent of injected dose per organ (% ID/ organ). At 4 h a. a. the biodistribution was as follows: 2.8 $\pm$ 0.1; 13.8 $\pm$ 2.4; 6.4 $\pm$ 0.2 % of injected dose in blood, liver and kidneys. The urinary excretion is accounted for 54.6% $\pm$ 1.7 at 4 h. Both thighs were dissected and counted and bacterial or sterile inflammation thigh / contralateral thigh ratio was then evaluated. At 4 h the ratios were 2.2 $\pm$ 0.4, 2.0 $\pm$ 0.4 and 2.3 $\pm$ 0.2 respectively for SA, EC and TURP. At 24 h, the ratios were 2.9 $\pm$ 0.9, 2.2 $\pm$ 0.7, and 4.1 $\pm$ 0.6 respectively. Imaging studies of mice were acquired at 4 and 24 h after administration. In all the animals the bacterial and sterile abscess were clearly visible.
In conclusion, the low *in vitro* ^{99m}Tc-CIP binding to bacterias observed and the localization of ^{99m}Tc-CIP in both infection (SA, EC) and sterile inflammation (TURP) could suggest that more work needs to be done to clearly document the exact mechanism and specificity of ^{99m}Tc-CIP localization at the site of infection.

Communication (accepted) to: "*International Symposium on Modern Trends in Radiopharmaceuticals for Diagnosis and Therapy*", Lisboa, 30 Março-3 Abril 1998.

Computacional Chemistry and Metal-based Radiopharmaceuticals

M. Neves[1] and R. Fausto[2]
[1]Departamento de Química. Instituto Tecnológico e Nuclear, 2685-Sacavém, Portugal
[2]Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3049-Coimbra, Portugal

Computational chemistry plays a fundamental role in molecular modeling of radiopharmaceuticals based on metal ions, as, Tc, In, Ga and lanthanides. In particular, in the case of Tc-radiopharmaceuticals, the prediction of the molecular structures of the Tc-complexes is particular relevant, since the amount (10^{-6}-10^{-8} M) of the radioactive ^{99m}Tc radiochemical species experimentally acessible, does not allow any inorganic classic chemical characterization and the corresponding ^{99}Tc chemistry is not always straightforward. Then, computer-assisted studies in these species, supported by both the available informations on the specific pecularities of the ^{99}Tc and/or Re chemistry and the previously determined molecular structures of the ligands, appear as an unique and very powerfull technique to further our understanding of the chemical reactivity and fundamental features of Tc-radiopharmaceuticals. In addition, the computer simulations of intermolecular interactions of these systems with biologically relevant molecules or aggregates, could also be used to recognize putative binding sites and thus help to understand and interpret *in vitro* and *in vivo* experiments, at a molecular level.
In this communication, a general overview of the most important computer-assisted techniques currently used is presented, with emphasis given to molecular mechanics and density-functional methods. In addition, the computer optimized geometries of oxo Tc-biguanides complexes are shown and compared with the available X-ray crystallographic data as well as those of a series

of analogues systems. Besides, a detailed computer-assisted evaluation of the main intermolecular interactions between the studied Tc-biguanides complexes and biochemically relevant molecules as water or simple peptide chains, is also presented and discussed. Finally, a series of important correlations between interaction energies and surface areas of the low-energy Tc-biguanides aggregate species and the results of *in vitro* studies (as partition coefficients and human protein binding of the ^{99m}Tc-biguanides) are established.
*Acknowledgements:*This work is finnancially supported by the PRAXIS XXI reseach projects QUI/2/2.1/412/94, SAU/2/2.1/1396/95 and FEDER.

Communication (accepted) to: "*International Symposium on Modern Trends in Radiopharmaceuticals for Diagnosis and Therapy*", Lisboa, 30 Março-3 Abril 1998.

Current Work

The number of biologically active peptides and their molecular weight confer to these molecules the desirable pharmacologic and pharmacokinetic properties to be used as starting points for radiopharmaceutical development. Recently, the application of small radiolabelled biologically active peptides for external imaging of a variety of biological processes has received considerable interest. An important difficulty is to combine the ability to radiolabelled these compounds with high specific activity using short-lived radionuclides, particulary ^{99m}Tc while maintaining high affinity binding to the receptor site.

Radiolabelling of peptides with ^{99m}Tc

C. Fernandes[1], R. Dias[1], L. Patrício[1], R. Moreira[2]
[1]ITN, Estrada Nacional nº10, 2685 Sacavém
[2]Fac.de Farmácia, Univ. de Lisboa, Av.das Forças Armadas, 1600 Lisboa

In the present study we are developing a novel approach for labelling peptides, with ^{99m}Tc which consists in attempt to synthesized oxotechnetium complexes with the functionalized monodentate thiol peptide derivative and a tridentate dithiol ligand. We intent to extend the acquired experience for labelling biologically active peptides.
The following work was developed: *S*-Benzoylthioglycolic acid was synthesized from thioglycolic acid and benzoic acid chloride, as described in the literature, in 87% yield. The activation of the carboxylic acid group through *N*-hydroxysuccinimide in the presence of dicyclohexilcarbodiimide (DCC) gave the succinimidyl-*S*-benzoylthioglycolate, in a 72% yield. This is a storable activated ester, which was coupled with a variety of aminoacids and peptides (glycine, sarcosine and their ethyl esters, glicyl-glycine, alanyl-glycine, and phenylalanyl-glycine). The conjugated compounds obtained were isolated, purified and characterized by determination of melting point, elemental analyses (C,H,N,S), I.R. spectra and ^{1}H NMR spectroscopy.
The conjugates of glycine, glicyl -glycine, and phenylalanyl- glycine were labelled with ^{99m}Tc. The method involves the use of the weak ^{99m}Tc-tartarate (Sn) complex previously prepared. ^{99m}Tc-tartarate (Sn) complex was added to the protected *S*-benzoyl conjugated peptides. The mixture was left at optimized experimental condition (alkaline medium, 100ºC for 15 min.) for cleavage of the protecting group. The thiolated peptide derivative formed was then

radiolabelled by transchelation from the weak tartarate complex in the presence of the tridentate ligand (2-mercaptoethyl sulfide). The radiochemical behavior was assessed by the following systems: TLC-silica gel with two different eluants: a) ethyl acetate, b) butanol, metanol, water and ammonia (60-20-20-1) and by ITLC-SG using acetone and NaCl 0.9% as eluants. The analysis of the radioactive profiles obtained indicated the presence of different radioactive species in all the radiolabelled compounds.
The octanol/saline partition of ^{99m}Tc-amino acid and/or peptides was also determined. The following results were obtained: 0.99±0.04, 0.021±0.002, 0.046±0.002 for glycine, glicyl-glycine and phenylalanyl-glycine respectively.
Biodistribution studies will be performed and a correlation between biodistribution and radiochemical properties established.

Radioiodination of Tyr-RC-160

L. Gano, L.Patrício
ITN, Estrada Nacional nº10, 2685 Sacavém

Radioiodination of Tyr-RC-160, an analogue of somatostatine, were accomplished by iodogen method. Under the optimized experimental conditions (pH, molar ratio peptide:oxidant, purification method) a high labelling efficiency was obtained (70-80%). The chemical and radiochemical analysis by reversed phase HPLC, have shown that the radioiodinated peptide with a low specific activity, and radiochemical purity higher than 95% is stable at least for 1 month.
The characteristics of radioiodinated peptide were evaluated by: receptor binding studies, lipophilicity and biodistribution. The binding affinity was studied by incubating rat brain cortex membrane fractions with increasing concentrations of labelled ligand in the absence of unlabelled ligand. The preliminar data were obtained. Further experiments will be done to established the true specific binding. A octanol/0.9% NaCl partition coefficient of 7.0 was obtained indicating that iodinated peptide has a high lipophilic character. The biodistribution data in mice 30 and 120 min after iv administration have shown a high uptake in the liver and intestines which is in agreement with the lipophilic characteristics of the compound. The high dose in stomach that increases at 120 min after administration indicated the *in vivo* instability of the iodinated peptide. The iodinated peptide will be use as reference for comparing ^{99m}Tc-labelled to be developed.