Radiopharmaceutical Development

The aim of the work done in this group has been the synthesis, radiochemical and biological characterization of potencial new radiopharmaceuticals and the assessment of their chemical structure/ biological activity relationship.

The most relevant activities which had been developed in this group, under the supervision of Luciana Catela Patrício until October 1999, included the development of:

- Radiolabelled receptor-binding radiopharmaceuticals based on peptides and steroidal molecules for tumour scintigraphy namely:

- Development of ^{99m}Tc labelled somatostatin analogues with suitable radiochemical and biological behaviour to present clinical potential for imaging of tumours, specially of neuroendocrine origin that express somatostatin receptors.

- Evaluation of the potential value of 17α-[^{125}I]iodovinylestradiol derivatives as imaging agents for estrogen receptor-positive breast tumours by determination of lipophilicity, binding to serum proteins, *in vitro* binding affinity for the estrogen receptor and biological activity.

- Baboon evaluation of biguanide labelled with ^{99m}Tc as a potential renal imaging radiopharmaceutical.

- A molecular mechanics study of several biphosphonates interation with hydroxyapatite in order to correlate their molecular structure with their bone resorption potency.

- Evaluation of the interference of two 3-hydroxy-4-pyridinones derivatives in the typical biodistribution of ^{67}Ga-citrate in experimental animals and correlation with their chelating properties and chemical structure.

- Evaluation of the role of neuropeptide Y and melanocortin peptides in the regulation of the gonadotropic axis in the rat with emphasis to the identification of the receptor subtype implicated in this regulation.

Due to the interest of reinforcing the organic and inorganic synthetic expertise of this group a reorganization has been done with the creation of a new team involving also researchers from the inorganic and organometalic chemistry group.

17α-[^{125}I]Iodovinylsteroids Substituted at 7α: Investigation of a New Series of Iodine[^{125}I]-Labelled estrogens as Potential Imaging Agents for Estrogen Receptor-Positive Breast Cancers[*]*

L. Patrício, M. C. Melo e Silva, L. Gano, F. Marques, M. L. Sá e Melo[1], T. Thiemann[2], A. C. Santos[3]

Objectives

Synthesis of novel estrogen receptor high affinity biomolecules with potencial application in oncology as imaging agents for human breast tumours followed by the evaluation of their *in vitro* and *in vivo* biological behaviour and by the correlation of their chemical structure/ biological activity relationships.

Results

Some estradiol derivatives (3-methoxy-(17α,20E)iodovinyl-6-dehydroestradiol; 3-methoxy-17α-ethynyl-6-dehydroestra-diol; 3-methoxy-7α-allyl-17α-ethynylestradiol; 3-methoxy-17α-ethynyl estradiol and 3-methoxy-estradiol) previously synthesised and characterised in our laboratory [1,2] were studied in order to evaluate their binding affinity for the estrogen receptor (ER): The relative binding affinities (RBA) were determined in cytosol from the uteri of immature Sprague-Dawley rats by a competitive assay with [^{3}H]estradiol using the dextran-coated charcoal method [3]. The RBA is defined as 100 times the ratio between the competitor and unlabelled estradiol concentrations required for 50% competition to specific tritiated estradiol binding.

A plot of % B/B_0 vs log competitor concentration was drawn. The analysis of the competition curves indicated that with exception of 3-methoxy-(17α,20E)iodovinyl-6-dehydroestradiol all the compounds inhibited the specific binding of [^{3}H]estradiol to the rat uterine cytosol but the relative binding affinity was in every case lower than estradiol. Moreover, the presence of a 3-methoxy group in the estradiol frame strongly reduces the ER binding affinity, the introduction of a 6,7-double bond seems to increase the ER binding affinity and a 7α-allyl moiety apparently does not affect the binding to the estrogen receptor.

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Further work

The biological behaviour of the steroid derivatives so far studied suggests us its potential as suitable radioligands for the imaging of estrogen receptors. These findings lead us to extend this systematic study to other non-steroid biomolecules in order to evaluate their value as therapeutic or imaging agents for estrogen-positive human breast tumours.

[*] Funding: PRAXIS/PSAU/C/SAU/89/96, Jul 1997-Dec1999. (18×10^6 PTE).

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Development of ^{99m}Tc labelled somatostatin and evaluation of their radiochemical and biological behaviour*

L. Gano, L. Patrício

Objectives

The main goal of this project was the improvement of a suitable method for ^{99m}Tc labelling somatostatin analogues in order to be potentially used as an imaging radiopharmaceutical of somatostatin receptors while mantaining the peptide biological activity. Thus the effect of radiolabelling procedure upon their radiochemical behaviour, biodistribution and receptor targetting should be evaluated.

Results

Following the synthesis, characterization and radiolabelling with ^{99m}Tc of the conjugates of two somatostatin analogues, Tyr^3-octreotide and RC-160, with 6-hydrazino nicotinate (Hynic) high labelling efficiencies were achieved. Both ^{99m}Tc-peptides, were obtained at specific activities of 37-74 GBq/ μmole with high radiochemical purity. The *in vitro* and *in vivo* stability and biodistribution in mice studies initialized last year, with ^{99m}Tc-octreotide, proceeded during 1999 and were also performed for ^{99m}Tc-RC-160. *In vitro* stability was determined by reversed phase HPLC and celulose acetate electrophoresis. The studies indicated that both the ^{99m}Tc-peptides are stable in the presence of human serum up to 24 h and do not undergo significant degree (< 5%) of transchelation when challenged with an excess (100 and 1000-fold molar) of cysteine.

Biodistribution studies in mice injection demonstrated that ^{99m}Tc-Tyr^3-octreotide is rapidly cleared from blood and that the tracer is mainly excreted *via* the kidneys with low uptake by the hepatobiliary system. In oppposite the ^{99m}Tc-RC-160 presented a markedly higher liver uptake, well correlated to its higher lipophilic character, confering it a worst biological profile. Also a lower pancreas and adrenals uptake was found. However the radiolabelled peptide was also rapidly cleared from blood and presented a high percent of radioactivity excreted via the urinary tract. HPLC and electrophoresis analysis of murine serum samples, showed high *in vivo* stability for both ^{99m}Tc-peptides and the analysis of urine indicated that ^{99m}Tc-compounds are mostly excreted as non-metabolised form.

Pancreas, adrenals and intestines are known to be rich in somatostatin receptors. In order to get an insight on the mechanism involved in the ^{99m}Tc-octreotide uptake biodistribution studies were performed, simultaneously into separated animal groups: without any treatment and previously injected with an excess of unlabelled peptide. For comparative purposes identical specific tissue uptake studies were run with ^{111}In-octreotide, a successfully clinical used radiopharmaceutical to image tumours that express somatostatin receptors. Owing to the better biologic profile of ^{99m}Tc-octreotide the comparison with ^{111}In-octreotide was only carried out for this peptide. The biodistribution pattern of ^{99m}Tc-octreotide in the pre-treated group did not present significant differences excepting the intestine and pancreas where the radioactivity accumulation decreased indicating a selective tissue uptake. These data correlate well with the results obtained with ^{111}In-octreotide despite of its higher radioactivity clearance from most of the tissues. Nevertheless specific uptake in same organs (pancreas and intestine) also was identified. The lower uptake in pancreas and intestine observed in the animals previously injected with unlabelled peptide indicates that ^{99m}Tc-octreotide uptake is somatostatin receptor-mediated.

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Further Work

We intend to proceed our studies by the evaluation of the *in vitro* binding properties of the peptidic Hynic-conjugates to membrane receptors in the rat cortex and the biodistribution in tumor bearing animals.

* Funding – Contract IAEA- 8972/R0 Dec 95/ May 2000 (9.5×10^6 PTE).

Chelating Properties Towards Gallium and Biological Evaluation of Two *N*-substituted 3-Hydroxy-4-pyridinones*

M. A. Santos[1], R. Grazina[1], A. Q. Neto[1], G. Cantinho[2], L. Gano, L. Patrício

Objectives

The aim of the project was the development of gallium chelating agents as models for potential biomedical applications, determination of the main chelating properties of the synthetised ligands by analytical studies of the respective Ga(III) complexes and evaluation of their interference in the typical biological distribution of the ^{67}Ga-citrate in experimental animals to get information about the removal of ^{67}Ga from its favourite tissue uptake and correlate with the chemical nature of the administered chelate.

Results

Following the synthesis of two *N*-substituted 3-hydroxy-4-pyridinones (3'-aminopropyl, L^1 and 2'-carboxylethyl, L^2 derivatives), the determination of the *pKa* values and partitioning coefficients of the ligands and the stability constants of their Ga(III) complexes (studies carried out at IST) a set of *in vivo* studies was undertaken in female mice, at ITN. These studies involve mostly the biodistribution evaluation of ^{67}Ga-citrate when the two chelating agents were co-injected. Both chelators have shown to interfere in the typical biological behaviour of the ^{67}Ga-citrate in mice. L^1 enhanced the urinary excretion leading to an increased ^{67}Ga removal from the soft tissue while L^2 induced to a lower blood clearance with a pronounced bone uptake mainly at 48 h after injection suggesting that the ^{67}Ga-L^2 complex could have some potential interest as bone imaging agent. The radioactive profiles from ITLC analysis of mice urine samples, collected at sacrifice time indicated that the radioactivity in the urine is either associated to ^{67}Ga-citrate or ^{67}Ga-complex, depending on the ligand administered L^1 or L^2, respectively. These results also suggest a higher *in vivo* stability.

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Further Work

Since ^{67}Ga-citrate is used in the diagnosis of a series of bone diseases and the bone uptake of ^{67}Ga is increased by the co-injection of the L^2 ligand, this work will proceed by studying the potential value of the ^{67}Ga-L^2 complex as a radiopharmaceutical for diagnosis purposes.

* Funding: PRAXIS/OPCEX/C/QUI/85/96, Out 1997-Mar 2000.

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Synthesis and Characterization of Biguanide Complexes with Technetium*

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Objectives

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.

Results

Previous biodistribution studies in mice and rats, have shown renal uptake. Also dynamic gamma-camera studies with ^{99m}Tc-Big and ^{99m}Tc-DMSA (a radiopharmaceutical in clinical use) in rabbits and baboons 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 are significantly higher for ^{99m}Tc-DMSA than for ^{99m}Tc-Big. During last year was developed a Big Kit (lyophilized preparation) and tested in baboons. These studies have shown that ^{99m}Tc-Big has favourable practical and dosimetric features for renal imaging as an alternative to ^{99m}Tc-DMSA.

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Further work

The project have been closed in the last October.

* Funding. Contract PRAXIS/2/2.1/SAU/1396/95 (15 × 10^6 PTE).

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Radiopharmaceuticals for Bone Therapy: A Molecular Mechanics Study of Lanthanides Bisphosphonates Complexes*

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Objectives

To find a correlation between the structure of 13 bisphosphonates (BPs) and their known bone resorption inhibition, by molecular mechanics.

To screen potential radiolanthanides (Sm-153, Dy-165, Ho-166, Tm-170 and Lu-177) BPs complexes, by molecular mechanics.

To test computational studies by *in vitro* and *in vivo* experiments. This implies the radioisotope production evaluation of radiolanthanides by RPI, and the syntheses, characterization and *in vitro* and *in vivo* studies of radiolanthanides BPs complexes.

Results

Since March /1999 (starting project date) the results are:

Molecular modelling of 13 BPs using MNDO molecular orbital approximation scheme within MOPAC, using Cerius-2 software MSI and Silicon Graphics Workstation O2.

Evaluation of capability production by RPI (Sm-153, Dy-165, Ho-166, Tm-170 and Lu-177) in terms of theoretical maximum specific activities for continnous and/or 12 hours cycles. Samples were irradiated and the measured activities were in agreement with the theoretical calculations.

Syntheses and characterization of alandronate, pamidronate, neridronate (compared with standard samples supplied by a pharmaceutical company) and YM-529.

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Further work

Untill March 2001, to run the project as proposed.

* Funding Contract: PRAXIS/C/SAU/14036/1998 (13×10^6 PTE).

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Regulation of the gonadotropic Axis by NPY and melanocortin peptides

Paula Raposinho, Michel Aubert[1]

Objectives

Evaluation of the role of neuropeptide Y (NPY) and melanocortin peptides for the regulation of the gonadotropic axis in the rat with special attention to the identification of the receptor subtype implicated in this regulation.

Results

It has been shown that NPY has an inhibitory role on the reproductive axis of castrated male rats. Using NPY agonists with different selectivity for the different receptors subtypes, we shown that acute intracerebroventricular NPY injection inhibits LH secretion in castrated rats by Y5-receptor subtype mediation [1]. We also shown that chronic NPY, PYY_{3-36} and hPP (two NPY-Y5-receptor agonists) infusion (7 days) in intact male rats produced an important hypogonadism with a decrease in several parameters studied (pituitary, seminal vesicles and testis weight, testosterone, LH and FSH plasma levels), suggesting that the Y5 receptor subtype is involved in the inhibition of the gonadotropic axis by NPY [2,3]. On the other hand central administration of NPY stimulates LH release in sex steroid-intact rats. We shown that the NPY Y1-antagonist/Y4-agonist 1229U91 strongly stimulates LH and FSH release in normal rats [4]. The pure Y1 agonist (BIBO3304) did not stimulates LH, excluding a Y1-mediation and suggesting the Y4-receptor as the subtype implicated in the stimulatory effect of NPY in the regulation of gonadotropic axis.

As the melanocortins-MC4-receptor and NPY pathways probably interact in the control of feeding and NPY was suggested as a possible dowstream effector of melanocortins signalling we compared the obesity syndrome generated by a 7-day central infusion of either NPY or the MC4-R antagonist SHU9119. Both peptides generated a similar hyperphagia and obesity. By contrast NPY produced a severe hypogonadism but no significant effects on reproduction function were seen with SHU9119, suggesting divergent hypothalamic pathways regulating food intake and neuroendocrine functions [5].

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Further work

Verify that NPY and some Y5 agonists inhibits LH in castrated mice but not in castrated Y5-knockout mice, thus further demonstrating the role of Y5-Receptor in the inhibition of gonadotropic axis by NPY. Verify that 1229U91 estimulates LH in intact wild-type mice and Y1-Knockout mice, thus demonstrating the Y4 pathway of stimulation of gonadotropic axis. Analyse, *in vivo*, the role of the Cocaine and Amphetamine Regulated Transcript (CART), a neuroendocrine factor that *in vitro* mediates the stimulatory action of leptin on the reproductive axis.

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