DEPARTAMENTO DE ENGENHARIA E CIÊNCIAS NUCLEARES (DECN)

Ciências Químicas e Radiofarmacêuticas

RELATÓRIO DE ACTIVIDADES

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1. SCIENTIFIC RESEARCH (2015-2018)

The scientific work accomplished during 2015-2018 is part of the strategy of Radiopharmaceutical Sciences Group (RSG), from Centro de Ciências e Tecnologias Nucleares (C^2TN), on the design and preclinical evaluation of specific halogen- and metal-based radioactive tools for PET or SPECT Molecular Imaging and Targeted Radionuclide Therapy based on beta- and Auger electron-emitters

The current research activity is focused on the development of novel radiotracers targeted to diagnostic and therapy in Nuclear Medicine. So far, emphasis has been placed on the design of labeled biologically active molecules together with their *in vitro* stability and biological activity evaluation.

Following previous research and within the running projects the scientific activities carried out during the last three-year period (February 2015 to February 2018) have included:

Radioiodinated Acridine Orange Derivatives as DNA-Targeting Probes for Auger Therapy

Targeted radiotherapy, using internally emitted radiation, can be an attractive alternative to conventional therapies such as surgery, external radiotherapy and chemotherapy. Early work has clearly shown the extreme cellular toxicity of Auger electron-emitters and demonstrated the capability of suitably targeted Auger electron decays within the cell nucleus to produce double strand DNA breaks and cell death with virtually no damage to the surrounding cells. Radiopharmaceuticals based on Auger emitter radionuclides are anticipated to afford a highly selective targeted radiotherapy, since the emitted Auger electrons deposit their low energy within subcellular dimensions.

In recent years, Auger-emitting radionuclides clinically used for SPECT imaging (*e.g.* ^{99m}Tc, $^{123/125}$I, ^{67}Ga or ^{111}In) have also started to be envisaged for selective and targeted radiotherapy. ^{125}I has been one the most studied and several studies have been published dealing with the potential usefulness of ^{125}I-labeled compounds as Auger-emitting radiopharmaceuticals for cancer therapy. One strategy to ensure the

close proximity of the emitting radionuclide to the DNA double helix and a prolonged retention time in the cell is the use of DNA intercalators that, upon intercalation between the DNA base pairs, can carry the radionuclide to short (sub)nanometric distances to DNA and, therefore, induce augmented DNA damage.

Following our previous work with ^{99m}Tc(I) tricarbonyl complexes, novel ^{125}I-labelled compounds bearing an acridine orange (AO) intercalating unit were evaluated for DNA-targeted Auger therapy. Their ability to cause DNA chain breaks, the influence of the radionuclide in DNA damage and the effect of the distance between the AO moiety and the radionuclide were investigated. For comparative purposes, we have extended this study to ^{99m}Tc complexes structurally related to the ^{125}I-labeled AO derivatives. Both classes of compounds were able to induce DNA double strand breaks but the extent of DNA damage and the role of direct effects were shown to be strongly dependent on the linker between the Auger emitter and the AO moiety. The experimental data has been corroborated by docking and nanodosimetric studies. Most of the compounds display moderate to high tumour cell uptake with significant accumulation in the nucleus. Pre-clinical evaluation of these radiolabeled AO derivatives is currently underway.

One of the major requirements of systemic radiation therapy is the definition of suitable tumour-selective vehicles in order to avoid normal tissue toxicity. Thus, several targeting vehicles, including peptides or antibodies, are currently being explored to enhance the selectivity of the ^{125}I-labeled AO tracers.

Radioiodinated LXXLL-based peptides for breast cancer targeting

Tumours over-expressing estrogen receptors (ER) have a great impact in women health and welfare since breast cancer is one of the most common forms of cancer and ovarian cancer is the most lethal of the gynaecological cancer. Despite some progress in early detection and treatment, mortality is still high. Since one-third of breast cancer patients respond to hormonal treatment, drugs based on estrogen receptor ligands can be useful for diagnosis and oriented therapy of the disease. Regardless of the many advances that have been made in endocrine therapy of ER+ tumours, novel therapeutic strategies are still required. One interesting approach to radionuclide

therapy of tumours associated to over-expression of estrogen receptors is the use of ER binding ligands labelled with Auger emitters.

Following the encouraging radiochemical and biological results obtained in RSG with small LXXLL-based peptides (L=Leucine, X= any aminoacid) labelled with ^{111}In for targeting ER-expressing tumours, these peptides were further labelled with ^{125}I to compare the radiobiological effects induced by both Auger emitters, ^{111}In and ^{125}I, and to assess the most promising radiolabeled compound for radiotheranostics of hormone responsive cancers.

The ER peptide displaying the highest ER binding affinity has been successfully radioiodinated with ^{125}I by electrophilic substitution on the imidazole ring of histidine residues. The ability of the peptide to internalize in the nucleus of breast carcinoma tumour cell lines was demonstrated by internalization studies in MCF 7. This feature is encouraging for a potential application of the radioiodinated peptide for Auger therapy since iodine radioisotopes are among the most potent Auger emitters.

Preliminary biodistribution studies and evaluation of *in vivo* stability were undertaken in healthy CD-1 mice. Biodistribution data in MCF 7 xenografted BALB/c nude female mice suggested some specific accumulation of activity in tumour and ER rich organs like uterus and ovaries, highlighting the potential of the tracer for ER imaging. Radiobiological studies are still underway.

Labeling of antibody fragments towards clickable radioimmunoconjugates as theranostic agents for TEM-1 targeting

Tumour vascular markers are attractive targets for antibody-based diagnostic and therapy. Tumour endothelial marker (TEM-1/endosialin/CD248) is a tumour vascular marker that is highly expressed in multiple human carcinomas, such as lung, ovarian and breast cancers, and with minimal expression in normal adult tissue. The major goal of this running project is the design of multifunctional Terbium radioimmunoconjugates for TEM-1 targeting. Terbium (Tb) is an excellent candidate for theranostic applications possessing four clinically relevant radioisotopes which are particularly attractive for imaging and therapy: ^{149}Tb (α emitter), ^{152}Tb (β+ emitter), ^{155}Tb (γ emitter), and ^{161}Tb (β- and Auger emitter).

Aiming to select the best candidate for the design of clickable radioimmunoconjugates that can be applicable in the clinic as theranostic agents for TEM-1 targeting, a small panel of antibody fragments targeting TEM-1 were labeled with ^{125}I and their specific TEM-1 targeting ability has been evaluated. The development of such radioiodinated conjugates and the investigation on innovative strategies to improve their pharmacological behaviour will provide new insights for the development of Tb-based radiopharmaceuticals on which few studies have been reported so far.

Optimization of labeling conditions, evaluation of radiochemical stability and assessment of cellular uptake in TEM-1 positive and negative cells were undertaken for all the radioiodinated fragments. Further *in vitro* and *in vivo* studies will be carried out, including biodistribution and SPECT imaging in tumour-bearing mice, antitumor effects, radiotoxicity and mechanisms of cell death together with micro and nano-dosimetric studies.

Other scientific activities

Within the running projects and other collaborative initiatives several biomolecules (*eg.* peptides, proteins) and small biologically interesting small molecules were also synthesized, labeled with ^{125}I, and biologically evaluated for subsequent utilization as reference compounds in *in vitro* receptor binding assays.

The training of new research students on radioiodination procedures and on safe handling of radioiodinated compounds is also within the scope of my responsibility. My work responsibilities also include the management/maintenance of radioiodination facilities, namely radioiodine chemicals, dedicated equipment and disposal of radioiodine contaminated waste. It is also my duty the management/maintenance of the HPLC equipment dedicated to the analysis of ^{125}I-labelled compounds and the training of new users.

2. RESEARCH PROJECTS

Participation

Molecular and Nano Tools for Cancer Theranostics. FCT (EXCL/QEQ-MED/0233/2012) (team member, co-coordinator of task 3. Multifunctional Indium Complexes for Cancer Theranostics: Synthesis, Characterization and Biological Evaluation).

3. EDUCATION AND TRAINING

Student supervision and education

Andreia Figueiredo. "^{18}F-FES PET: Da química à clínica". Internship in Nuclear Medicine III. Graduation in Nuclear Medicine. Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa. Fevereiro-Junho 2015.

Carlota de Sousa. "Aplicação da terapia com radionuclídeos emissors de electrões Auger no carcinoma da prostate: ^{125}I-DCIBzL". Internship in Nuclear Medicine II. Graduation in Nuclear Medicine. Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa. Setembro-Dezembro 2015.

Andreia Neves. "Marcação de Fragmentos de Anticorpos com ^{125}I". Internship in Nuclear Medicine III. Graduation in Nuclear Medicine. Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa. Fevereiro-Junho 2017.

4. SCIENTIFIC REVIEW AND EVALUATION ACTIVITIES

Scientific review

Member of editorial board of referees of *Arkivok* (ARKAT, USA, Inc)

Invited reviewer for manuscripts submitted to: *Arkivok (2016),* Anti-Cancer Agents in Medicinal Chemistry *(2017)*

Participation in examining committees

MSc thesis of Edgar Pereira. "Compostos Radioiodados para Terapia Auger". Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, 30 outubro 2015. Jury member.

PhD thesis of Susana Cunha. "Avaliação do Potencial Clínico de Novos Ligandos para o Receptor Estrogénico em Diagnóstico e Terapia de Tumores da Mama". Faculdade de Ciências da Universidade de Lisboa, 22 Maio 2015. Jury member.

5. NATIONAL AND INTERNATIONAL SCIENTIFIC COLLABORATIONS

Professor Amélia P. Rauter. Carbohydrate Chemistry Group, FCUL, Lisboa, Portugal.

Professor Miguel Castanho, Unidade de Bioquímica Física, Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisboa, Portugal.

Professor Thies Thiemann, Department of Chemistry, Faculty of Science, United Arab Emirates University, United Arab Emirates.

6. PUBLICATIONS

6.1 Papers in international scientific periodicals with referees

1. S. Di Maria, A. Belchior, E. Pereira, L. Quental, M. C. Oliveira, F. Mendes, J. Lavrado, A. Paulo, P. Vaz. Dosimetry assessment of DNA damage by Auger-emitting radionuclides: Experimental and Monte Carlo studies. Radiation Physics and Chemistry, 140, 278-282 (2017) https://doi.org/10.1016/j.radphyschem.2017.01.028

2. M. Morais, H. Zamora-Carreras, P. D. Raposinho, M. C. Oliveira, D. Pantoja-Uceda, J. D. G. Correia, M. Jiménez. NMR Insights into the Structure-Function Relationships in the Binding of Melanocortin Analogues to the MC1R Receptor. Molecules, 22, 1189 (2017) https://doi:10.3390/molecules22071189

3. E. Pereira, L. do Quental, E. Palma, M. C. Oliveira, F. Mendes, P. Raposinho, I. Correia, J. Lavrado, S. Di Maria, A. Belchior, P. Vaz, I. Santos, A. Paulo. Evaluation of Acridine Orange Derivatives as DNA-Targeted Radiopharmaceuticals for Auger Therapy: Influence of the Radionuclide and Distance to DNA. Scientific Reports, 7, 42544 (2017) https://doi:10.1038/srep42544

4. E. Palma, F.Marques, L. Gano, M. C. Oliveira, A. Abrunhosa, H. V. Miranda, T. F Outeiro, I. Santos, A. Paulo. Synthesis and Biological Evaluation of Novel 2-Aryl Benzimidazoles as Chemotherapeutic Agents. Journal of Heterocyclic Chemistry, 54, 255-267 (2017) https://doi:10.1002/jhet.2575

5. Y. Al Jasem, T. Thiemann, L. Gano, M. C. Oliveira. Fluorinated steroids and their derivatives. Journal of Fluorine Chemistry, 185, 48-85 (2016) https://doi.org/10.1016/j.jfluchem.2016.03.009

6. M. C. Oliveira, L. Gano, I. Santos, J. D. G. Correia, I. D. Serrano, S. S. Santos, M. Ribeiro, J. Perazzo, I. Tavares, M. Heras, E. Bardaji, M. A. Castanho. Improvement of the pharmacological properties of amidated kyotorphin by means of iodination. MedChemComm, 7, 906-913 (2016) https://doi:10.1039/C6MD00028B

7. F. Vultos, S. Cunha, C. Fernandes, M. C. Oliveira, F. Marques, M. F. Botelho, I. Santos, L. Gano. New estradiol based ^{111}In complex towards the estrogen receptor. Radiochimica Acta, 103, 765-776 (2015) https://doi.org/10.1515/ract-2014-2355

8. P. Nunes, G. R. Morais, E. Palma, F. Silva, M. C. Oliveira, V. F. C. Ferreira, F. Mendes, L. Gano, H. V. Miranda, T. F Outeiro, I. Santos, A. Paulo. Isostructural Re (I)/99m Tc (I) tricarbonyl complexes for cancer theranostics. Organic & Biomolecular Chemistry, 13, 5182-5194 (2015) https://doi:10.1039/C5OB00124B

6.2 Papers in national scientific periodicals with referees

C. Rodrigues, M. C. Oliveira. Radioimunoterapia: uma abordagem terapêutica promissora no tratamento do carcinoma do ovário. Saúde & Tecnologia, 14: 26-35 (2015).

7. COMMUNICATIONS IN SCIENTIFIC MEETINGS

1. E. Pereira, L. do Quental, E. Palma, M. C. Oliveira, F. Mendes, P. Raposinho, I. Correia, J. Lavrado, S. Di Maria, A. Belchior, P. Vaz, I. Santos, A. Paulo. Acridine-Orange derivatives as DNA-targeted radiopharmaceuticals for Auger therapy. Proteção Radiológica na Saúde (PRS2017) September 27-29, 2017, IST, Lisbon, Portugal.

2. V. Cachatra, A. Martins, M. C. Oliveira, L. Gano, T. Man, D.Evans, M. Walter, N. Colabufo, A. P. Rauter. Unraveling purine nucleoside key structure for anticholinesterase and anticancer activities., European Carbohydrate Symposium (Eurocarb 2017) July 2-8, 2017, Barcelona, Spain.

3. A. D'Onofrio, L. Gano, M. C. Oliveira, J. Fierle, S. Dunn, A. Paulo. Towards clickable radio-immunoconjugates as theranostic agents for TEM-1 targeting. Encontro Ciência 2017. July 3-5, 2017. Centro de Congressos de Lisboa, Lisbon, Portugal.

4. F. Vultos, M. Belo, M. Scheepstra, C. Fernandes, M. C. Oliveira, F. Mendes, L. Brunsveld, J. D.G. Correia, I.Santos, L. Gano, Radiolabeled peptides targeting the estrogen receptor for breast cancer theranostics: ^{111}In- versus ^{125}I-labelling. J Label Compd Radiopharm 60 (Suppl. 1): S197 (2017). https://doi:10.1002/jlcr.3508 The 22nd International Symposium on Radiopharmaceutical Sciences (ISRS 2017), May 14-19, 2017, Dresden, Germany.

5. E. Pereira, A. Paulo, M. C. Oliveira, F. Mendes, P. Raposinho, A. Belchior, I. Correia, J. Lavrado. Radioiodinated compounds for Auger therapy. Eur J Nucl Med Mol Imaging 43 (Suppl 1): S433 (2016) https://doi:10.1007/s00259-016-3484-4 29th Annual Congress of the European Association of Nuclear Medicine (EANM '16), October 15-19, 2016, Barcelona, Spain.

6. J. Correia, L. Gano, I. Santos, I. D. Serrano, S. S. Santos, M. Ribeiro, J. Perazzo, I. Tavares, M. Heras, E. Bardaji, M. A. Castanho, M. C. Oliveira. Biological Evaluation of Radioiodinated Amidated Kyotorphin. Journal of Peptide Science, 22, (Suppl 2): S158-S158 (2016) https://doi:10.1002/psc.2950 34th European Peptide Symposium 4-9 September 2016, Leipzig, Germany.

7. E. Pereira, L. do Quental, M.C. Oliveira, P. Raposinho, A. Belchior, S. Di Maria, I. Correia, J. Lavrado, F. Mendes, P. Vaz, I. Santos, A. Paulo. Radiolabeled Acridine Orange (AO) Derivatives as DNA-Targeted Probes for Auger Therapy, Radiotherapy and Oncology. 118: S83 (2016), https://doi.org/10.1016/S0167-8140(16)30170-0 International Conference on Translational Research in Radio-Oncology and Physics for Health (ICTR-PHE) 2016 February 15-19, CICG, Geneva, Switzerland.

8. V. Cachatra, S. Schwarz, M. C. Oliveira, L. Gano, A. Paulo, A. P. Rauter. Targeting Butyrycholinesterase for New Treatments in Alzheimer's Disesase and Cancer. CQB-Day 2015, Faculdade de Ciências, Universidade de Lisboa, September 2015, Lisbon, Portugal.

9. V. Cachatra, S. Schwarz, M. C. Oliveira, L. Gano, A. Paulo, A..P. Rauter. Butyrylcholinesterases as a target for degenerative disease therapies. 19th European Symposium on Organic Chemistry (ESOC 2015), July 12-16, 2015, Lisbon, Portugal.

10. F. Vultos, M. Belo, C. Fernandes, J. D. G. Correia, M. C. Oliveira, I. Santos, L. Gano. Influence of the radionuclide on the stability and biological profile of a ER

targeting peptide. Workshop LOWDOSE-PT- 2015, Biological effects and risks of low dose and protected exposures to ionizing radiation, April, 2015, Bobadela, Portugal

11. S. Cunha, F. Vultos, C. Fernandes, F. Marques, M. C. Oliveira, I. Santos, L. Gano. ^{111}In- and ^{67}Ga-estradiol based complexes for breast cancer imaging: radiochemical and biological evaluation. Workshop LOWDOSE-PT- 2015, Biological effects and risks of low dose and protected exposures to ionizing radiation, April, 2015, Bobadela, Portugal (**oral**)

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Pólo de Loures - Campus Tecnológico e Nuclear, 27 de Fevereiro de 2018