REPORT

2009


iMed.UL
Research Institute for Medicines
and Pharmaceutical Sciences

Contents

1. Unit Description

1. UNIT DESCRIPTION

1.1 Institutional

The Institute for Medicines and Pharmaceutical Sciences (iMed.UL) is a non-profit research centre at the Faculty of Pharmacy, University of Lisbon (FFUL) that brings together scientists mainly from the FFUL, as well as the National Institute for Engineering, Technology and Innovation (INETI), Lisbon.

1.2 Mission

The mission of iMed.UL is to foster cutting-edge research at the interface of biology, chemistry and pharmaceutical sciences with the aim of understanding the genetic and molecular basis of human diseases, to identify molecular targets and mechanisms, and to translate these into the design of target-selective molecules and delivery systems for tomorrow's diagnostics and therapeutics. Advanced training in pharmaceutical and related sciences at the postgraduate level is also one of iMed.UL's core activities

1.3 Structure and management

Coordinator: Rui Moreira (elected in January 2010)
Coordination Council: Group Leaders
Scientific Council: researchers holding a PhD degree
At present, research programmes and projects are organized in seven transversal scientific areas, each one corresponding to a major research group coordinated by a senior scientist. An internal set of rules and a protocol with FFUL establishes how the Unit operates internally and in its relationship with the host institution. An external Scientific Advisory Board includes scientific personalities that have the mission of auditing and perform recommendations on the research activities, goals and attainable achievements: Leslie Benet (UCSF/School of Pharmacy, USA), Ruth Duncan (Cardiff Univ./Welsh School of Pharmacy, UK), David Stevenson (Stanford Univ. School of Medicine, USA), Ferran Sanz (Univ. Pompeu Fabra, Barcelona, Spain), Per Spindler (Univ. of Copenhagen, Denmark), Charles Hoppel (Case Western Reserve Univ., USA); Pierluigi Nicotera (Univ. of Leicester, UK).

1.4 Research Groups | Coordinators

Molecular and Biology of Eukaryotic Systems | Cecília Maria Pereira Rodrigues
Neuron Glia Biology in Health and Disease | Dora Maria Tuna de Oliveira Brites
Metabolism and Genetics | Maria Isabel Ginestal Tavares Almeida
Chemical Biology and Toxicology | Matilde da Luz dos Santos Duque da Fonseca e Castro
Medicinal Chemistry | Rui Ferreira Alves Moreira
Nanomedicine and Drug Delivery Systems | Rogério Paulo Pinto de Sá Gaspar
Pharmacological Sciences | Maria Beatriz Silva Lima

2. Objectives

2. OBJECTIVES

Medicines play a foremost role in Society. As science progresses, knowledge in Biomedicine and Pharmaceutical Sciences provides a plethora of new pharmaceuticals that are instrumental in improving public health beyond expectations. However, our therapeutic approaches still need major improvement, while further progress constantly requires extra efforts on basic and applied research. In Europe, Drug Discovery and Development (DDD) are lagging behind other world regions and tremendous investments are now required and already planned.

DDD clearly requires a multidisciplinary/integrative approach. iMed.UL gathers several research groups within Medicinal Products development and Pharmaceutical Sciences focused on innovation, thus contributing and being part of the European endeavour in the field. The groups congregate proven experience, skills and critical mass in several domains that cover drug research, from target identification and drug design to basic signalling mechanisms and drug delivery, including nanomedicine approaches, as well as in-use surveillance.

The Unit research programme forms the basis of general approaches that cover different steps of DDD. Target identification in neurodevelopmental, neurodegenerative and orphan diseases addresses the medical needs of early and late age populations, by focusing on genetic defects and mechanisms that mediate specific processes of cellular dysfunction and disrupted metabolic pathways. This encompasses novel diagnosis, investigation of molecular regulatory processes of cell death and survival, with its multiple implications from cell degeneration and proliferation to stem cell biology, and studies of alterations resulting from exposure to common neurotoxic conditions in perinatal life bridging neonatal brain deficits and mental disorders.

Other biological processes and pathological conditions are currently investigated including oxidative stress, inflammation, oncology, as well as infectious and auto-immune diseases.

Drug design based on molecular modelling and drug delivery based on nano and micro systems underlie the therapeutic approaches proposed. To this end, molecular modelling will be used, including novel chemical scaffolds from natural sources and subsequent selective and efficient rational synthetic strategies, as well as macromolecular complexation and particle engineering to provide organ and cell delivery or improve bioavailability of poorly absorbed drugs, including proteins for replacement therapy.

Comprehensive intervention in related fields is an asset in DDD. Therefore the Unit will address risk assessment by studying mechanisms of xenobiotic toxicity as well as development and validation of molecular biomarkers for use in predictive toxicology. Studies on cellular mechanisms involved in inflammation and male reproductive function using molecules intended to be developed as therapeutic candidates will be complemented with the use of in silico tools to predict the pharmacokinetics of potential new drugs.

Safe and effective use of medicinal products is a major concern in public health. Activity in this area is one of the Unit's responsibilities through consistent programs in pharmacoepidemiology, population pharmacokinetics and bioequivalence assessment. Finally, transversal research projects across several groups have already been launched and will be one of the main endeavours of this novel R&D Institute.

3. Activities

3. ACTIVITIES

3.1 Integrative/ multidisciplinary activities:

Several activities were undertaken during this period, which involved integration and interaction between several groups. These included:

1. Exchange of training and research activities involving in vitro and in vivo assays as well as technologies and biotechnological processes (e.g. protein purification, enzyme assays, cytotoxicity assays and pharmacokinetic studies in animals) that allowed a clear improvement in collaborative papers, joint project submissions, and advanced training;
2. Joint applications for funding in FCT projects that include several iMed.UL groups and international partners. These projects involve graduate students as well as young members of staff, including researchers hired from Ciência 2007/2008 Program. In addition, these projects offer a training interface between Chemistry, Nanotechnology, Toxicology and Biology (see individual group sections for details);
3. Cultured mammalian cell systems and signalling pathways investigated by Mol Cell Biol and NeuronGlia groups were applied to the assessment of apoptotic/anti-apoptotic/anti-oxidative/anti-inflammatory effects of new synthetic compounds and biotech products provided by MedChem and ChemBiolToxicol groups, respectively. Integrative activities originated publications in international peer reviewed journals, presentations in international meetings, and submission of concerted projects;
4. Met&Gen and Nano/DDS Groups have been collaborating in the nanoencapsulation of a stable form of the recombinant hPAH protein for development of a new PKU therapeutic approach. This activity was consolidated by the approval of a FCT project, a Ph.D. scholarship and the publication of a peer reviewed paper;
5. Shared training and supervision of Master and PhD students as well as postdocs, some of which have already successfully concluded their degree. A major outcome of this effort towards an integrative strategy was the financial support from FCT of 11 contracts within Ciência 2007/2008 that are expected to strengthen the interface areas of iMed.UL;
6. Seminars were promoted regularly during 2009 (total of 25, 8 of which by international speakers) that were open to the University. Most seminars were part of post-graduate programs.

3.2 Outreach activities

iMed.UL was strongly involved in organizing schools, post-graduate courses, workshops and seminars, as well as meetings and activities aimed at bridging science and the general public and schools. In addition, iMed.UL collaborated with both national and international organizations in activities within the Medicinal Product sector, as well as professional, scientific and educational societies. The outreach activities included:

School visits and scientific training

Open laboratories and visits of scientists to schools organized within the International Brain Awareness Week by the International Dana Alliance for Brain Initiatives and by the Portuguese Society for Neurosciences; Scientific training of high school students in holidays, through the project

"Ocupação Científica de Jovens nas Férias", in collaboration with Ciência Viva; Professional laboratory training of high school students to encourage participative relationships with universities;

Organization of schools and post-graduate courses

Post-graduate course “Biology of Cell Death I: Mechanisms of Apoptosis”, 6th Edition, Lisbon; Research workshop “Monitoring Cell Death”, Lisbon; Post-graduate course “Actualização às Metodologias de Avaliação da Inflamação”, Lisbon; Post-graduate course “Inflammation, an experimental approach”, Lisbon;

Organization of symposia

International symposium “Sociedade Portuguesa de Doenças Metabólicas (SPDM)”, Curia, October; International symposium “BioEM09: International meeting on Biomedical Electron Microscopy Applications” http://www.ff.ul.pt/paginas/jvitor/BIOEM09/Bioem09/Welcome.html; International symposium “A Collaborative Solution on Tropical Diseases: A Triennial Initiative in Partnership with NIAID/NIH" http://www.fladtropicaldiseases.com/; European Science Foundation-UB Second European Summer School in Nanomedicine, http://www.ff.ul.pt/nanoschool2009/.

Participation in the board of directors of scientific societies and organizations

ERA-NET Nanomedicine (External Advisory Board); European Research Institute for Integrated Cellular Pathology (founding membership); Bilirubin Translational Research Committee; Society for the Study of Inborn Errors of Metabolism; European Metabolic Group (Advisory Board); European Federation for Pharmaceutical Sciences (Executive Committee and Board); International evaluation panel of CIBER-Spain (Chairmanship); European Federation for Medicinal Chemistry (Council Member); Sociedade Portuguesa de Neurociências; Sociedade Portuguesa de Hepatologia; Sociedade Portuguesa de Ciências Farmacêuticas; Sociedade Portuguesa de Doenças Metabólicas;

Collaboration or participation in specialized committees:

European and Portuguese Medicines Agency (EMEA and INFARMED); European Platform for Clinical Tests on AIDS/HIV, malaria and tuberculosis; Scientific advice (interaction with patient groups, involvement in program setting for new medicinal products development and approval for Europe, multipartite discussion and training initiatives involving academia, pharmaceutical industry and regulators) on pharmacokinetics and pharmacotoxicology; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH); European Cooperation in Science and Technology (COST) actions

4. General Indicators

4. GENERAL INDICATORS

4.1 Funding

	2008	2009
Unit FCT	341351.00	369187.50
Projects FCT	631900.00	408005.52
Other (National)	282500.00	269178.60
Other (International)	27200.00	0.00
National Industry	48700.00	25000.00
International Industry	0.00	26255.17
Total	**1331651.00**	**1097626.79**

4.2 Human Resources

	2005	2006	2007	2008	2009
Total No. of Researchers (FTE)	61	66	82	97	102
No. of Researchers Hired (Ciência Programme)	--	--	--	6	4

4.3 Training and publication output in 2005-2009

	2005	2006	2007	2008	2009	Total
Training Masters (Master thesis completed)	6	6	17	22	16	67
Training PhDs (PhD thesis completed)	6	10	4	9	9	38
Publications in International Journals	42	47	60	78	103	330

5. Publications

5. PUBLICATIONS | 2009

5.1 Peer-reviewed journals

1. Alfaia CPM, Alves SP, Martins SIV, Costa ASH, Fontes CMGA, Lemos JPC, Bessa RJB, Prates JAM. Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. Food Chem. 2009: 114: 939-946. (IF = 3.146).
2. Alverca E, Andrade M, Dias E, Bento FS, Batoréu MCC, Jordan P, Silva MJ, Pereira P. Morphological and ultrastructural effects of microcystin-LR from Microcystis aeruginosa extract on a kidney cell line. Toxicon. 2009, 54: 283-294. (IF = 2. 437).
3. Almeida R, Cabral Marques H. Optimisation of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes. Eur J Pharm Biopharm. 2009, 73: 121-129. (IF= 3.151).
4. Amaral JD, Castro RE, Steer CJ, Rodrigues CMP. p53 and the regulation of hepatocyte apoptosis: implications for disease pathogenesis. Trends Mol Med. 2009, 15: 531-541. (IF = 11. 049).
5. Amaral JD, Solá S, Steer CJ, Rodrigues CMP. Role of nuclear steroid receptors in apoptosis. Curr Med Chem. 2009, 16: 3886-3902. (IF = 4. 708).
6. Amaral JD, Viana RJS, Steer CJ, Rodrigues CMP. Bile acids: regulation of apoptosis by ursodeoxycholyc acid. J Lipid Res. 2009, 50: 1721-1734. (IF = 4. 917).
7. Amaro MI, Rocha J, Vila-Real H, Eduardo-Figueira M, Mota-Filipe H, Sepodes B, Ribeiro MH. Anti-inflammatory activity of naringin and the biosynthesised naringenin by naringinase immobilized in microstructured materials in a model of DSS-induced colitis in mice. Food Res Int. 2009, 42: 1010-1017 (IF = 2. 414).
8. Amat M, Gómez-Esqué A, Escolano C, Santos MMM, Molins E, Bosch J. Enantioselective Formal Synthesis of (+)-Dihydrocorynantheine and (—)-Dihydrocorynantheol, J Org Chem. 2009, 74: 1205-1211. (IF = 4. 219).
9. Aranha MM, Solá S, Low WC, Steer CJ, Rodrigues CMP. Caspases and p53 modulate FOXO3A/Id1 signaling during mouse neural stem cell differentiation. J Cell Biochem. 2009, 107: 748-758. (IF = 2. 935).
10. Ascenso A, Marques H C. Acne in the Adult. Mini-Rev Med Chem. 2009, 9: 1-10. (IF = 2. 971).
11. Bignotto L, Rocha J, Sepodes B, Eduardo-Figueira M, Pinto R, Chaud M, de Carvalho J, Moreno H Jr, Mota-Filipe H. Anti-inflammatory effect of lycopene on carrageenan-induced paw edema and hepatic ischemia-reperfusion in the rat. Brit J Nutr. 2009, 102: 126-133. (IF = 3. 446).
12. Brachkova MI, Duarte A, Pinto JF. Evaluation of the viability of Lactobacillus spp. after the production of different solid dosage forms. J Pharm Sci. 2009, 98: 3329-3339. (IF= 2. 906).
13. Brites D, Fernandes A, Falcão AS, Gordo AC, Silva RFM, Brito MA. Biological risks for neurological abnormalities associated with hyperbilirubinemia. J Perinatol. 2009; 29: S8-S13. (IF = 1. 593).
14. Borralho PM, Kren BT, Castro RE, da Silva IBM, Steer CJ, Rodrigues CMP. microRNA-143 reduces viability and increases sensitivity to 5-fluorouracil in HCT116 human colorectal cancer cells. FEBS J. 2009, 276: 6689-6700. (IF = 3.042).
15. Cabral-Marques H, Almeida R. Optimisation of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes Eur J Pharm Biopharm. 2009. 73: 121–129. (IF= 3. 151).

16. Caldeira RL, Gonçalves LMD, Martins TM, Silveira H, Novo C, do Rosário V, Domingos A. Plasmodium chabaudi: expression of active recombinant chabaupain-1 and localization studies in Anopheles sp. Exp Parasitol. 2009, 122: 97-105. (IF = 1.773).

17. Candeias NR, Branco LC, Góis PMP, Afonso CAM, Trindade AF. More Sustainable Approaches for the Synthesis of N-Based Heterocycles. Chem Rev. 2009, 109: 2703-2802. (IF = 35. 957).

18. Candeias NR, Veiros LF, Afonso CAM, Gois PMP. Water: A Suitable Medium for the Petasis Borono-Mannich Reaction. Eur J Org Chem. 2009: 1859-1863. (IF = 3.096).

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21. Cascalheira JF, João SS, Pinhanços SS, Castro R, Palmeira M, Almeida S, Faria MC, Domingues FC. Serum homocysteine: interplay with other circulating and genetic factors in association to Alzheimer's type dementia. Clin Biochem. 2009, 42: 783-790. (IF = 2. 019).

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27. Correr CJ, Pontarolo R, Wiens A, Rossignoli P, Melchiors AC, Radominski R, Fernandez-Llimós F. Economic evaluation of pharmacotherapeutic follow-up in type 2 diabetes mellitus patients in community pharmacies. Arq Bras Endocrinol. 2009, 53: 825-833. (IF = 0.680)

28. Costa E, Vieira E, dos Santos R, Lopes AL, Saldanha MJ, Brites D. Identification of a novel deletion in UDP-glucuronosyltransferase gene in a patient with Crigler-Najjar syndrome type I. Blood Cells Mol Dis. 2009, 42: 265-266. (IF = 2. 901).

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5.2 Books and book chapters

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6. Seminars

6. **iMed.UL | Seminars 2009**

22. Jan. 09 | **Iola Duarte**, CICECO, Universidade de Aveiro | The role of NMR-based metabonomics in disease diagnosis and drug development | Host: Rogério Sá Gaspar

20. Feb. 09 | **Miguel Xavier Fernandes**, Universidade da Madeira | Introduction of selectivity in the design of lead compounds using molecular modeling techniques | Host: Rui Moreira

13. Mar. 09 | **Paula Alexandra Quintela Videira**, Medical Sciences Faculty, UNL | Glico-Imunologia: o papel do ácido siálico | Host: Maria de Jesus Perry

27. Mar. 09 | **Pedro L. Granja**, INEB, Instituto de Engenharia Biomédica, Portugal | Lab-made organs and tissues | Host: Matilde Castro

03. Apr. 09 | **Tiago Outeiro**, Cellular and Molecular Neuroscience, Unit at the IMM | From Molecules to Systems: Deciphering the Molecular Basics of Neurodegeneration | Host: Cecília Rodrigues

17. Apr. 09 | **Teresa Garcia**, APDI e Oficina de Transferência de Tecnol., Universidade de Lisboa, Portugal | Patentes e transferência de tecnologia | Host: Mafalda Ascensão

24. Apr. 09 | **Graça Soveral**, COFB – FCT/UNL (Laboratório Ass. Requimte) | Looking into Membrane Transport and Regulation: from nanovesicles to tubular organs | Host: José Morais

08. May. 09 | **João Bettencourt Relvas**, IBMC, Porto | Extracellular regulation of myelination | Host: Dora Brites

15. May. 09 | **Maria Mota**, Instituto de Medicina Molecular ! Approaching malária from the host side | Host: Cecília Rodrigues

22. May.09 | **Daniele Del Rio**, Department of Public Health, University of Parma ! Polyphenols and health. Thrown off track by the usual suspects | Host: Maria Bronze

29. May. 09 | **Ana Oliveira Brett**, Departamento de Química, Universidade de Coimbra | Biossensores electroquímicos com DNA: caracterização da superfície por AFM e aplicações para a detecção electroquímica in situ de danos oxidativos causados ao DNA | Host: M. Camila Batoréu

05. Jun. 09 | **António Castanheira**, Serviço de Toxicologia Forense, Delegação do Sul do Instituto Nacional de Medicina Legal | General approach of analytical method validation in Forensic Laboratories | Host: Catarina Dias

29. Jun. 09 | **Debra Roter**, Jonhs Hopkins University, USA | How to develop the pharmacy service? The Roter Interaction Analysis System (RIAS) as a fresh perspective to improve pharmacist-patient interaction | Host: Afonso Miguel Cavaco

03. Jul. 09 | **Paulo Pereira**, Centro de Oftalmologia, IBILI – Faculdade Medicina da Univesidade de Coimbra | A new route for degradation of HIF-1a and endothelial dysfunction in diabetes | Host: Maria João Gama

10. Jul. 09 | **Peter Vandenabeele**, University of Gent, Belgium | Apoptotic and non-apoptotic functions of caspases, an overview | Host: Cecília Rodrigues

22. Jul. 09 | **Luis García Río**, Departamento Química – Física, Faculdade de Química, Universidade de Santiago de Compostela | Utilización de Microemulsiones como Nuevos Medios de Reacción | Host: António Calado

18. Sep. 09 | **António Paulo**, Instituto Tecnológico e Nuclear | Technetium Organometallic Complexes for Myocardial Imaging: From Bench to Bedside | Host: Rui Moreira

25. Sep. 09 | **Anita Gomes**, Escola Superior de Tecnologias da Saúde | Identification of tumor-associated antigens involved in the anti-lymphoma activity of gd T cells | Host: Rita Castro

28. Sep. 09 | **Larry Mcreynols**, Senior Scientist, New Englad Biolabs, Inc. USA | Deep Sequencing of Small RNAs from the Parasitic Nematode *Brugia malay* | Host: Jorge Vítor

02. Oct. 09 | **Pedro Viana Baptista**, Centro de Investigação em Genética Molecular Humana (CIGMH), Departamento de Ciências da Vida, FCT-UNL | BioNanoTechnology for nanodiagnostics | Host: Matilde Castro

09. Oct.09 | **Gonçalo J. L. Bernardes**, Departament of Chemistry, University of Oxford |Therapeutic Proteins: Tools for Synthetic Biology | Host: Pedro Góis

20. Nov. 09 | **Joana Palha**, Life and Health Sciences Research Institute (ICVS), University of Minho | he choroid plexus: a site for signaling in and out of the brain | Host: Alexandra Brito

27. Nov. 09 | **Belén Pérez**, Centro de Biologia Molecular Severo ochoa (CBMSO), Universidad Autónoma de Madrid | RNA- Based therapy in inherited metabolic diseases | Host: Isabel Tavares de Almeida

27. Nov. 09 | **José Miguel Pêgo**, Instituto de Investigação em Ciências da Vida e da Saúde da Universidade do Minho | Influência do *stress* na estrutura e função da amígdala | Host: Dora Brites

04. Dec. 09 | **Richard Morgan**, New England Biolabs | Engineering novel DNA binding specificity in Type II restriction endonucleases | Host: Jorge Vítor

7. Research Groups

7. RESEARCH GROUPS

7.1 Molecular and Cell Biology of Eukaryotic Systems

Objectives

Several specific aims have been designed to accomplish our main goal of investigating molecular regulatory aspects involved in cell death, proliferation and differentiation, with its multiple implications in crucial biological processes, from neurobiology to cancer and stem cell biology.

Modulation of cell death and proliferation by bile acids

Further explore the antiapoptotic versus proliferative functions of bile acids and determine the role of microRNAs (miRNAs). This includes the screen of specific cell death and proliferative pathways and investigation of the potential involvement of miRNAs in liver regeneration and colorectal cancer.

Role of apoptosis in neurodegeneration, neuroprotection and neurogenesis

Further characterize the role of apoptosis in neurodegeneration using cell systems as well as pharmacologic and transgenic animal models of disease, and determine the effect of antiapoptotic bile acid treatment. Dissect the cross-talk between mechanisms of protein aggregation and apoptotic cell death. In addition, explore the impact of genetic diversity of drug metabolizing enzymes and other polymorphic genes on the individual responses to environment, specifically in age-associated neurodegenerative diseases. Finally, investigate whether the apoptosis machinery is required for differentiation of neural stem cells, and if modulation of apoptosis mediates stem cell self-renewal.

Regulatory pathways that control cholesterol homeostasis in the brain

Characterize molecular mechanisms that underlie phenotypic expression of drug metabolizing enzyme genes and their adaptive responses to external stimuli, as models of mammalian gene regulation. Further elucidate the relevance of mechanisms of cholesterol homeostasis in chronic neurodegeneration.

The results are expected to advance our understanding of basic mechanisms of disease, as well as to contribute for the development of novel therapeutic targets and strategies for disorders of imbalanced cell death and survival.

Principal investigator

Cecília M. P. Rodrigues, Full Professor FFUL

Research team

Elsa Rodrigues, Assistant Professor FFUL
Isabel B. Moreira da Silva, Assistant Professor FFUL
Maria João Gama, Assistant Professor FFUL
Susana Solá, Assistant Professor FFUL
Margarida Castro Caldas, Assistant Professor FCT-UNL
Rui E. Castro, Assistant Investigator (Ciência 2008)
Ana Filipa Nunes, Postdoc FCT
Joana D. Amaral, Postdoc FCT
Pedro M. Borralho, Postdoc FCT
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Duarte M. S. Ferreira, PhD student
Inês Milagre, PhD student
Márcia M. Aranha, PhD student
Maria Benedita Fonseca, PhD student
Maria João Nunes, PhD student
Ricardo J. S. Viana, PhD student
Joana M. Xavier, BI fellow

Main achievements

Neurodegeneration and neural differentiation

Cell death plays a significant role in the pathogenesis of neurodegenerative disorders. Thus, it is crucial to elucidate basic mechanisms of cell function that may culminate in cell death, and develop effective therapeutic strategies. In this regard, our main achievements were:

Elucidation of regulatory mechanisms of human CYP46A1 transcription by demonstrating the importance of an epigenetic program, namely the role of histone acetylation (Nunes et al., 2009, accepted).

Clarification of evidence that dissociates the pro-apoptotic properties of amyloid β peptides from their distinct mechanisms of aggregation/fibrillization in vitro, providing new perspectives for modulation of amyloid toxicity (Viana et al., 2009).

Demonstration that Gstpi is actively expressed under oxidative stress in C57BL/6 mice brain, mostly in oligodendrocytes and astrocytes (Castro Caldas et al., 2009a), and directly interacts with JNK in neuronal cells (Castro Caldas et al., 2009b).

Demonstration of safety, tolerability and cerebral spinal fluid penetration of ursodeoxycholic acid in patients with amyotrophic lateral sclerosis (Parry et al., 2009, accepted

The engagement and activity of apoptotic pathways may favor either cell death or differentiation. We have shown that apoptosis-associated factors such as caspases and p53 temporally modulate FOXO3A/Id1 signaling and differentiation of mouse neural stem cells (Aranha et al. 2009).

Liver degeneration and regeneration

In the recent past, we have discovered that ursodeoxycholic acid, an endogenous bile acid, is a strong inhibitor of apoptosis that modulates cell death and cell cycle proteins. We have previously established the p53/Mdm-2 association as a prime target for bile acid modulation of p53-mediated cell death in hepatocytes (Amaral et al., 2007), with no evidence of direct binding between ursodeoxycholic acid and the p53 DNA-binding domain (Amaral et al., 2009, in press). Ursodeoxycholic acid was also shown to modulate the ubiquitin-proteasome degradation pathway of p53 (Amaral et al., submitted). Further, toxic and non-toxic bile acids are known to differentially regulate cyclin D1 and cell death in hepatocytes (Castro et al., 2007). Finally, we have identified microRNA profiles during rat liver regeneration after partial hepatectomy and characterized modulation of miRNA expression by ursodeoxycholic acid (Castro et al., 2009, submitted).

Colon cancer biology

miRNAs play a significant role in the pathogenesis of cancer and response to chemotherapy. We have performed highly sensitive miRNA analyses and identified stage, mismatch repair status specific miRNA expression and partial reversion to stem cell like signature in colorectal cancer (Sarver et al., 2009). We have also shown in the past that inhibition of Fas expression by RNAi modulates 5-

fluorouracil-induced apoptosis in p53-proficient human colon cancer cells (Borralho et al., 2007). Importantly, we have explored microRNA-143 biological role and demonstrated that it reduces viability and increases sensitivity to 5-fluorouracil in HCT116 human colorectal cancer cells, signaling through ERK5/NF-kB pathway(s) (Borralho et al., 2009).

We have published 13 papers and presented 44 communications. We have provided advanced training to 9 graduate students (1 PhD and 1 MSc theses concluded), organized a Postgraduate Course and Research Workshop (total, 34 students), and participated in 3 Master programs, including the Master/PhD in Neurosciences (Faculty of Medicine), and two in-house Master courses. We have collaborated within iMed.UL (two original joint publications) and with national and international groups. We have coordinated five funded research projects. Finally, we have been involved in several outreach activities within the International Brain Awareness Week, and Ciência Viva Programs.

Publications

Amaral JD, Castro RE, Steer CJ, Rodrigues CMP. p53 and the regulation of hepatocyte apoptosis: implications for disease pathogenesis. Trends Mol Med. 2009, 15: 531-541. (IF = 11. 049).

Amaral JD, Solá S, Steer CJ, Rodrigues CMP. Role of nuclear steroid receptors in apoptosis. Curr Med Chem. 2009, 16: 3886-3902. (IF = 4. 708).

Amaral JD, Viana RJS, Steer CJ, Rodrigues CMP. Bile acids: regulation of apoptosis by ursodeoxycholyc acid. J Lipid Res. 2009, 50: 1721-1734. (IF = 4. 917).

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Sarver AL, French AJ,Borralho PM, Thayanithy V, Oberg AL, Silverstein KAT, Morlan BW, Riska SM, Boardman LA, Cunnigham JM, Subramanian S, Wang L, Smyrk TC, Rodrigues CMP, Thibodeau SN, Steer CJ. Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states. BMC Cancer. 2009, 9: 401. (IF = 2.736).

Viana RJS, Nunes AF, Castro RE, Ramalho RM, Meyerson J, Fossati S, Ghiso J, Rostagno A, Rodrigues CMP. Tauroursodeoxycholic acid prevents E22Q Alzheimer's A beta toxicity in human cerebral endothelial cells. Cell Mol Life Sci. 2009, 66: 1094-1104. (IF = 6.090).

Master and PhD thesis

PhD thesis

Pedro M. Borralho. Role of apoptosis and miRNAs in identifying novel therapeutic targets in colon cancer. University of Lisbon, Portugal, 2009.

Master Thesis

Mauro Vecchi. Role of apoptosis-associated pathways in neural stem cell differentiation. Universita' Degli Studi di Milano-Bicocca, Italy, 2009.

Invited lectures and seminars

Rodrigues CMP. Apoptosis: game and players. Portuguese-British Meeting on Modulation of Programmed Cell Death and Therapeutic Targets in Cancer and Neurodegenerative Diseases, Porto, Portugal, 2009.

Castro RE, Rodrigues CMP. Mecanismos da doença hepatica alcoólica. II Congresso Português de Hepatologia, Sociedade Portuguesa de Hepatologia, Lisboa, Portugal, 2009.

Rodrigues CMP. The emerging role of bile acids in modulating neurodegeneration. Encontro Ciência 2009 do Conselho de Laboratórios Associados e da Fundação para a Ciência e a Tecnologia, Lisboa, Portugal, 2009.

Castro RE, Rodrigues CMP. Tumores primitivos do fígado: Aspectos genéticos e moleculares. III Congresso Português de Hepatologia, Sociedade Portuguesa de Hepatologia, Évora, Portugal, 2009.

Organization of conferences

Laboratory visits and conferences *Porque morrem os neurónios?* International Brain Awareness Week, Dana Alliance for Brain Initiatives and Portuguese Society for Neurosciences, in collaboration with Ciência Viva, 2009.

Laboratory work and conferences *Porque morrem os neurónios?* Ocupação Científica de Jovens em Férias, in collaboration with Ciência Viva, 2009.

2nd Portuguese Congress of Hepatology, Portuguese Society of Hepatology, Lisbon, Portugal, 2009.

Ongoing projects

Neurodegeneration in Alzheimer's disease transgenic mice: role of apoptosis and its modulation (PTDC/SAU FCF/67912/06). FCT 07-10. PI: CMP Rodrigues

Estrogen signaling and epigenetics modifications involved in the brain-specific expression of the CYP46A1 gene (PTDC/SAU-GMG/64176/06). FCT 07-10. PI: E Rodrigues

Linking fibrillogenesis and the apoptosis induced by amyloid β mutant peptides (PTDC/BIA-BCM/67922/06). FCT 08-11. PI: CMP Rodrigues

Understanding microRNA profiles and cellular functions in colorectal cancer (PTDC/SAU-GMG/099162/08). FCT 09-12. PI: CMP Rodrigues

Exploring new roles for endogenous bile acids and microRNAs in modulating liver regeneration (PTDC/SAU-OSM/102099/08). FCT 09-12. PI: RE Castro

Role of miRNAs in hepatic regeneration (Soc Port Gastro 2008). SPG 09-11. PI: CMP Rodrigues.

7.2 Neuron Glia Biology in Health and Disease

Objectives

The research within the Neuron Glia Biology in Health and Disease unit concentrates on the molecular and cellular analysis of fundamental brain functions, neurodegenerative and neuroregenerative processes and aging, as well as on the development of the brain. Our work programmes also encompass disturbances of the central nervous system (CNS) function secondary to loss of blood-brain barrier properties and liver dysfunction. The research should translate into innovative targeted drug delivery strategies and new therapeutic approaches for CNS diseases. The specific objectives for each of our 5 research programmes for 2009 were:

Cellular neurosciences, CNS injury and repair

Evaluate glial cell reactivity following challenge with unconjugated bilirubin or cytokines addressing: (i) the susceptibility of precursor oligodendrocytes using new primary culture models; (ii) the mechanisms underlying microglia activation; (iii) how sepsis may aggravate bilirubin-induced neurotoxicity in young cells.

Explore neuron-glia interplay using co-cultures systems neuron/astrocyte to evaluate the protective or the aggravating role of astrocytes on induced neurotoxicity.

Dissect whether mitochondria is a first target to cell dysfunction following unconjugated bilirubin and/or cytokines challenge and explore related cell death mechanisms.

Encephalopathy associated to liver dysfunction

Encephalopathy may be determined by an accumulation of toxic compounds in the blood as a result of liver damage. Hepatic glucuronidation of bilirubin is catalyzed by UGT1A1, which is essential for its efficient biliary excretion. Genetic alterations causing absence, or reduction, of UGT1A1 enzymatic activity result respectively in Crigler–Najjar and Gilbert syndromes. The former syndrome leads to bilirubin encephalopathy. Thus, additionally to the diagnosis of the syndromes, we are exploring UGT1A1 polymorphisms and mutations that may determine these familial unconjugated hyperbilirubinemias in collaboration with the Unidade de Genética Molecular, Porto (Rosário dos Santos e Elísio Costa).

Knowledge on the combined effects of bilirubin and bile acids in liver dysfunction and consequent brain damage is scarce so we investigated whether fetal hepatocytes are more vulnerable than adult ones when facing anicteric chronic cholestasis, conjugated or unconjugated hyperbilirubinemia, or their association. This is important because chronic hepatic diseases may determine liver failure and secondary brain damage, in which newborns may show increased vulnerability.

Neurodevelopment: stages, susceptibilities and outcomes

Based on previous data suggesting that unconjugated bilirubin impairs neurite outgrowth, the objectives were to evaluate whether it also affect: (i) differentiation of neuronal precursors; (ii) neuronal development and establishment of synapses; and if so (iii) whether alterations of cytoskeleton dynamics are involved.

Neural stem cell proliferation is associated with increased levels of oxidative stress. Therefore, the profile of the oxidative status along neural stem cell proliferation and differentiation was determined.

During neuronal development neurite elaboration is influenced by many factors, including the availability of guidance cues including Netrin1. So we explored the effects of Netrin 1 on the

differentiation of embryonic hippocampal neurons and establishment of dendritic and axonal arbors. In addition, most of the tracing of neuronal images to define the number and lengths of the neurites in several studies is performed manually and the collected data also analyzed cell by cell. Hence, to obviate time consuming posttracing data manipulation we will develop an automated analysis of tracing data.

Blood brain barrier biology and modulation

The in vitro BBB model coupled with various technological platforms and co-culture configurations has become a powerful mainstay tool for studying CNS drug pharmacokinetics, CNS therapeutic targeting, neuroinflammation, neurodegeneration, neuroprotection, and neurotoxicity. Therefore, a human and rat blood brain barrier models to assess loss of function by septic and jaundiced conditions will be established.

Therapeutics for CNS disorders

Based on our previous results indicating that glycoursodeoxycholic acid has anti-inflammatory, anti-oxidant, and anti-apoptotic effects, we will continue to explore whether this bile acid may also prevent: (i) neurotoxicity following unconjugated bilirubin exposure in co-culture systems; and (ii) loss of blood brain barrier function.

In intra-iMed.UL collaborative studies we will explore the neuroprotective efficacy of natural (e.g. naringin and naringenin) or synthetic compounds in our research models.

Principal investigator

Dora Maria Tuna de Oliveira Brites, PhD (Senior Researcher, Invited Full Professor)

Research team

Maria A. Brito, Assistant Professor FFUL
Rui F. M. Silva, Assistant Professor FFUL
Maria L. Correia, Assistant Professor FFUL
Adelaide Fernandes, Assistant Researcher (Ciência 2008)
Ana S. Falcão, Postdoc FCT
Ana R. Vaz, PhD Student FCT
Sandra L Silva, PhD Student FCT
Andreia Barateiro, PhD Student FCT
Eduarda Coutinho, Master Student
Catarina Vizetto Duarte, Master Student
Ema Torrado, Master Student
Filipa Cardoso, Master Student
Inês Palmela, Research Fellow
Catarina Osório, Research Fellow
Cibelle Mariano, Master Student
Sonia Loreto, Master Student
Andreia Ferreira, Master Student
Maria Pedro Pereira, Master Student
Sandra Sousa, Master Student
Maria Elisa Monteiro Micaelo Alves, Graduate Technician

Main achievements

Cellular neurosciences, CNS injury and repair.

Two reviews and one book on the response of astrocytes, neurons and microglia to unconjugated bilirubin implicating the cascade of events and the role of each nerve cell type and maturation state were published (Brites et al., J Perinatol. 2009; Fernandes and Brites, Curr Pharm Des. 2009; Brites D et al. Encyclopedia of Neuroscience, Springer). We implemented primary rat cultures of oligodendrocyte precursor cells and showed that bilirubin triggers mitochondrial dysfunction and ER stress (oral presentation, Barateiro A et al., iMed.UL Meeting). Following our data on the release of cytokines by microglia, upon exposure to bilirubin, we further showed that earlier phagocytosis is followed by inflammatory response with the activation of MAPKs, NF-κB and COX-2 (2 oral presentations, Silva et al., Trieste Yellow Retreat, iMed.UL Meeting and preparation of a manuscript). Pro-inflammatory cytokines increase the expression of nNOS and NO production, and the activation of extrinsic and intrinsic apoptosis by bilirubin (Vaz AR et al., manuscript in preparation). In immature neurons bilirubin alters mitochondrial respiratory chain, depletes oxygen consumption, and increases glycolysis and extracellular ATP (Vaz AR et al., J Neurochem, accepted; work in collaboration with A Almeida and J Bolanos,University of Salamanca). Astrocytes when in co-culture aggravate neuronal damage by bilirubin (Falcão et al., manuscript in preparation).

Encephalopathy associated to liver dysfunction

Our group performs unique studies in the country related with bilirubin and bile acid profiles in jaundice and cholestasis. We described a novel deletion in the UGT1A1 gene in a patient with Crigler–Najjar syndrome type I (Costa E et al., Blood Cells Mol Dis. 2009). The work received 2 awards in 2009 as the Best Clinical Case in National Meetings (Congresso de Gastrenterologia and APEF). Evaluation of the reactivity of human fetal and adult immortalized hepatocytes to bilirubin and bile acids, either alone or in association indicated that the poor prognosis exerted by bilirubin in chronic cholestasis is due to the marked toxicity of these molecules together, literally destroying hepatocytes (Duarte CV, Master Thesis, 2009; oral presentation and abstract at the XXIX Congresso Nacional de Gastrenterologia e Endoscopia Digestiva).

Neurodevelopment: stages, susceptibilities and outcomes

In neurosphere cultures bilirubin impairs neurogenesis without affecting gliogenesis. In hippocampal neurons, decreases the extension of neuronal arborization and the synaptic density (Fernandes A et al. Dev Neurobiol. 2009). Changes in the expression and localization patterns of MAP2 and Tau1, and in kinesin and dynein were observed (Coutinho E, Master Thesis, 2009). Netrin 1 increases secondary branches on both axons and dendrites, but not axon length, dendrites, or branches either in poly-D-lysine or laminin. Analysis by the XL_Calculations program, developed by us, showed to decrease time consumption and to facilitate morphometric analysis (Popko J et al., Cytometry A. 2009; work in collaboration with L Lanier, University of Minnesota). Unique characteristics of stem cells are related to redox status. We observed an increased concentration of total glutathione with a high Mrp1 expression in the proliferating state when compared to the differentiating one. Bilirubin aggravated oxidative stress in the first stages of nerve cell differentiation (Torrado E, Master Thesis, 2009; manuscript in preparation; work in collaboration with D Henrique (IMM) and C Tiribelli (CSF, Trieste).

Blood brain barrier (BBB) biology and modulation

The BBB strictly controls the exchanges between blood and brain compartments playing a key role in CNS homeostasis. We have established in vitro models of both human (Bernas MJ et al., Nat Protoc, accepted) and rat BBB cultures. We showed that bilirubin and pro-inflammatory cytokines promote

the apoptosis of the endothelial cells (work in collaboration with M Bernas of the University of Arizona, KS Kim of the Johns Hopkins University School of Medicine, and both Y Persidsky and SH Ramirez of the Temple University School of Medicine.

Therapeutics for CNS disorders

Our studies with glycoursodeoxycholic have demonstrated its ability in preventing: (i) the release of cytokines by astrocytes (observed for IL-10 as well) (Fernandes and Brites, Curr Pharm Des. 2009); (ii) aggravation of neurotoxicity by astrocytes (manuscript in preparation); (ii) mitochondria dysfunction (Vaz AR et al., J Neurochem, accepted); and (iii) endothelial cell death following bilirubin exposure (see above). In our intra-iMed.UL collaborative studies, we have showed that naringin and naringenin are effective anti-inflammatory agents preventing LPS-induced astroglial inflammatory response. Naringenin demonstrated a higher efficacy than indomethacin.

We published 3 original papers, 2 reviews and 1 book chapter, and presented 29 communications. We provided advanced training to 2 Post-doctoral, 3 PhD and 4 Master students, organized one Postgraduate course and one Workshop on the experimental approaches to assess inflammation, and participated in 4 Master courses - three at FFUL and in Neurosciences (IMM/FML). We coordinated three funded research projects and collaborated in projects within iMed.UL or with national and international groups. We have also participated in outreach activities.

Publications

Brites D, Fernandes A, Falcão AS, Gordo AC, Silva RFM, Brito MA. Biological risks for neurological abnormalities associated with hyperbilirubinemia. J Perinatol. 2009; 29: S8-S13. (IF = 1. 593).

Brites D, Fernandes A, Falcão AS, Brito MA, Silva RFM.Glial and Neuronal Reactivity to Unconjugated Bilirubin. In: Binder MD, Hirokawa N, Windhorst U, Hirsch MC (eds) Encyclopedia of Neuroscience. Neuroimmunology. Springer, Heidelberg, Germany. 2009 : 1726-1730.

Costa E, Vieira E, dos Santos R, Lopes AL, Saldanha MJ, Brites D. Identification of a novel deletion in UDP-glucuronosyltransferase gene in a patient with Crigler-Najjar syndrome type I. Blood Cells Mol Dis. 2009, 42: 265-266. (IF = 2. 901).

Fernandes A, Brites D. Contribution of inflammatory processes to nerve cell toxicity by bilirubin and efficacy of potential therapeutic agents. Curr Pharm Design. 2009, 15: 2915-2926. (IF = 4.414).

Fernandes A, Falcão AS, Abranches E, Bekman E, Henrique D, Lanier LM, Brites D. Bilirubin as a determinant for altered neurogenesis, neuritogenesis, and synaptogenesis. Dev Neurobiol. 2009, 69: 568-582. (IF= 2. 732).

Popko J, Fernandes A, Brites D, Lanier LM. Automated analysis of NeuronJ tracing data. Cytom Part A. 2009, 75: 371-376. (IF = 3.032).

Master and PhD thesis

Master thesis

Filipa Cardoso. Establishment and characterization of a human model of the blood-brain barrier. Master graduation in Molecular Genetics and Biomedicine, Faculty of Sciences and Technology, New University of Lisbon, Portugal, 2009.

Ema Torrado. Effect of unconjugated bilirubin on nerve cells development: role of Mrp1. Master graduation in Molecular Genetics and Biomedicine, Faculty of Sciences and Technology, New University of Lisbon, Portugal, 2009.

Catarina Vizetto Duarte. Reactivity of fetal and adult hepatocytes to bile acids: from cytokine production to MAPKs and NFkappaB signalling. Master thesis in Molecular Genetics and Biomedicine, Faculty of Sciences and Technology, New University of Lisbon, Portugal, 2009.
Eduarda Coutinho. Neuronal cytoskeletal dynamic modification induced by unconjugated bilirubin. Master graduation in Human Molecular Biology, Faculty of Sciences and Technology, New University of Lisbon, 2009.

Invited lectures and seminars

Silva RFM, Silva SL, Brito MA, Falcão AS, Fernandes A, Vaz AR, Brites D. The yellow brain: neurotoxic actions of unconjugated bilirubin. Seminar at Biotechnical Faculty, Department of Food Science and Technology, Ljubljana, Slovenia, 2009.
Leitão Silva S, Vaz AR, Barateiro A, Falcão AS, Fernandes A, Brito MA, Silva R, Brites D. Microglia: a double-edged sword when bilirubin gets into the brain. Trieste Yellow Retreat 2009, Trieste, Italy, 2009.
Vaz AR, Esteban MD, Brito MA, Bolaños JP, Brites D, Almeida A. Bilirubin selectively inhibits cytochrome c oxidase leading to energy impairment and apoptotic death in immature cortical neurons. Trieste Yellow Retreat 2009, Trieste, 2009.
Vaz AR, Delgado-Esteban M, Brito MA, Bolaños JP, Almeida A, Brites D. Energy impairment, oxidative stress and apoptotic death occurs in undifferentiated neurons exposed to bilirubin and may be prevented by glycoursodeoxycholic acid. 1st Post- Graduate iMed.UL Students Meeting, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, 2009.
Silva SL, Vaz AR, Falcão AS, Fernandes A, Barateiro A, Brito MA, Silva RFM, Brites D. Cellular and molecular reactivity of microglia to unconjugated bilirubin. 1st Post-Graduate iMed.UL Students Meeting, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, 2009.
Barateiro A, Vaz AR, Silva SL, Fernandes A, Brites D. Unconjugated bilirubin mediates oligodendrocyte precursor cell death by multiple pathways. 1st Post-Graduate iMed.UL Students Meeting, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, 2009.

Organization of conferences

Postgraduate course on Inflamação: Uma Abordagem Experimental, Faculty of Pharmacy, University of Lisbon, January 26-30, 2009.
Workshop on Actualização às Metodologias de Avaliação da Inflamação, Faculty of Pharmacy, University of Lisbon, February 19-21, 2009.

Ongoing projects

Acute and long-term injury of bilirubin to nerve cells - Looking for sensors, susceptibilities and modulators (PPCDT/SAU-MMO/55955/2004), FCT 07-09, 15 K€. PI: Dora Brites
Uncovering the potential neurodevelopmental deficits in common neonatal conditions such as hyperbilirubinemia and inflammation (PTDC/SAU-NEU/64385/2006), FCT 07-10, 99K€. PI: Dora Brites
Study of the blood-brain barrier disruption by neurotoxins and modulation by therapeutic agents in a human model: effects of bilirubin and endotoxin on brain microvascular endothelial cell monolayer integrity and protective role of bile acids (PTDC/SAU-FCF/68819/2006), FCT 07-10, 120K€. PI: Maria A Brito
Importância do perfil dos ácidos biliares séricos na avaliação da disfunção hepática" (ACS/PAF/49/158), Alto Comissariado da Saúde 08-09, 41K€ PI: Dora Brites

Trophic actions of neurotrophic factors: dependency of adenosine A2A receptors co-activation (PTDC/SAU-NEU/64126/2006), FCT 07-10, 13K€. PI:Ana Sebastião (collaborative Project)
Estrogen signaling and epigenetic modifications in the regulation of the CYP46A1 expression in the brain (PTDC/SAU-GMG/64176/2006), FCT 07-10. PI: Elsa Rodrigues (collaborative Project).

7.3 Metabolism and Genetics

Objectives

In line with the ongoing research activity and to unravel the raised questions our objectives will be focused on:

Mitochondrial Dysfunction / Energy metabolism / Inborn Errors of Metabolism (IEM)

The impact of VPA inhibition on CPT1A will be further characterized, using rat CPT 1A expressed in S. cerevisiae. The hypothesis that CPT1A sensitivity to malonyl-CoA could be primarily affected will be investigated. The mechanisms of hyperammonemia, underlying the interference of VPA and VP-CoA with the urea cycle, will also be elucidated at the level of the synthesis of N-acetylglutamate (NAG) either in vitro or in vivo;

Characterisation of the effect of mFAO intermediates on PDHc activity; Study of the potential inhibitory activity of trans-2-enoyl-CoAs upon CPT2 activity; functional/Structural characterisation of the E.coli expressed 6xHis_hCACT_Strep protein; functional characterisation and subcellular localization of the SLC25A45 protein, homologous to CACT-like protein (SLC25A29) and CACT, in a yeast expression system;

Unravelling the mechanisms responsible for the testis-specific expression of PDHA2 gene: identification of cis-acting sequences and respective transcription factors and elucidation of the potential epigenetic control induced by DNA methylation and histone deacetylation.

Misfolded proteins / Protein stabilization / IEM

Encapsulation, using nanobiomaterials (alginate and chitosan), of the stabilized hPAHwt enzyme, an essential pre-step for the design of further animal studies;

Extending the study of the role of chemical chaperones in the modulation of the folding pathway and stabilization of other mutant proteins involved in IEM, namely PDHc and Cystathionine-β-Synthase;

Apply the developed analytical strategy (bicistronic system) to study interallelic complementation in IEM.

Homocysteine / Endothetial Dysfunction / IEM

Study the effect of the accumulation of the Hcy precursor (AdoHcy, S-adenosyl homocysteine) on NO bioavailability, unravelling the underlying molecular mechanisms;

Identification of genetic determinants of hyperhomocysteinemia, mild forms.

Principal investigator

Maria Isabel Ginestal Tavares Almeida, PhD

Research team

Fátima Vieira Ventura, Assistant Professor FFUL
Isabel Rivera, Assistant Professor FFUL
Margarida Fernandes Baptista Silva, Assistant Professor FFUL
Maria João Silva, Assistant Professor FFUL
Paula Leandro, Assistant Professor FFUL
Rita Castro, Assistant Professor FFUL
Margarida Santos Leite, Invited Professor FFUL
Henriqueta Marques dos Santos, Assistant Professor FFUL
Ana Gaspar, MD HstaM / FMUL

Ana Isabel Coelho, PhD Student FCT
Ana Pinheiro, PhD Student FCT
Cátia C. P. Aires, PhD Student FCT
João Leandro, PhD Student FCT
Marisa Simas Mendes, PhD Student FCT
Mónica Rocha, PhD Student FCT
Paula B. M. Luís, PhD Student FCT
Paulo Roque Lino, PhD Student FCT
Ruben Esse, PhD Student FCT
Sandra Brasil, PhD Student FCT
Sara Violante, PhD Student FCT
Fábio Madeira, BI Fellow
Madalena Barroso, BI Fellow
Ruben Ramos, BI Fellow
Andreia Luz, Master Student
Cristina Florindo, MsC Student
Dina Mendes, BII Fellow
Francisca Lopes, BII Fellow
Israel Gonçalves Junior, BII Fellow FFUL
Rita Mexia, BII Fellow FFUL

Main achievements

Mitochondrial Dysfunction / Energy metabolism / IEM

We characterized the mechanism underlying the effect of both VP-CoA and Δ4-VP-CoA, on the hepatic isoform of CPT1, using rat CPT 1A expressed in S. cerevisiae. It was clearly demonstrated that VP-CoA inhibits CPT1A, through a specific interference at the catalytic domain of the enzyme. The inhibitory role of malonyl-CoA was clearly affected, resulting in an increase of the Ki for malonyl-CoA, with the increase of valproyl-CoA concentration (Aires CCP et al, Biochem Pharmacol, 2009, ePub ahead of print);

The hepatic levels of N-acetylglutamate (NAG) were significantly reduced in rats treated with VPA as compared with control tissues. This quantification was achieved using LC-MS/MS, after adequate development of the analytical method;

Demonstration that the yeast expressed human CPT2 is competitively inhibited by trans-2-enoyl-CoAs (paper under revision);

Preliminary results on functional characterisation of the yeast expressed mouse SLC25a45 protein, homologous to CACT-like protein (SLC25A29) and CACT, revealed that it is not an carnitine/acylcarnitine transporter;

Treatment of a human neuroblastoma cell line (SH-SY5Y) with 5-aza-deoxycytidine (AZA, a DNA methyltransferase inhibitor) and trichostatin A (TSA, a histone deacetylase inhibitor) revealed that the promoter CpG island is insensitive to demethylation while the exonic CpG island was around 50% demethylated. Transcription levels were evaluated by qPCR for PDHA2 and PDHA1 (its somatic paralogous) genes: in control cultures PDHA1 displayed normal transcriptional levels and PDHA2 displayed minimal levels; after 96 hours of treatment with AZA and TSA, PDHA1 maintained the same transcriptional levels, while PDHA2 revealed significantly increased transcriptional levels;

Moreover, transient transfection of mammalian cells with several PDHA2 reporter constructs revealed high luciferase activities in somatic cells where PDHA2 mRNA could not be detected and functional deletion analysis of the human PDHA2 gene promoter identified several activator and a

repressor binding regions. Preliminary results show that Sp1 transcription factor binds to the promoter and must be implicated on PDHA2 gene transcription mechanism.

Misfolded proteins / Protein stabilization / IEM

Preliminary results demonstrated that, although in low yields, a functional form of recombinant hPAH was successfully nanoencapsulated;

Enzymatic characterization of hybrid forms of hPAH showed that interactions between the different mutant subunits occur, influencing the kinetic properties of the heteroallelic protein and contributing to genotype/phenotype inconsistencies and BH4-nonresponsiveness in some compound heterozygous PKU patients (paper submitted);

Using the expression systems developed in our group, we successfully produced functional forms of: (a) α and β subunits of human E1 PDH protein (bicistronic system) and; (b) the Cystathionine-β-Synthase protein (pET system) in high yields and purity grade.

Homocysteine / Endothetial Dysfunction / IEM

We elucidated the effect of the Hcy precursor (AdoHcy) on NO bioavailability in cultured human endothelial cells, which we found to be significantly impaired;

We unraveled the molecular basis of the phenotype above described assessing the effect of AdoHcy on (a) the activity of NOS3; PRMT1; DDAH2 and Cav1 genes, both at transcriptional and translational levels; (b) eNOS activity and (c) caveolin 1 cell compartmentalization (paper under preparation);

We also focused on the study of the genetic determinants of Hcy, confirming that the TCN2 776C>G genotype exerts a significant influence upon B12 cellular delivery, but not on Hcy plasma levels (Castro R et al. Clin Biochem. 2010, in press).

Publications

Cascalheira JF, João SS, Pinhanços SS, Castro R, Palmeira M, Almeida S, Faria MC, Domingues FC. Serum homocysteine: interplay with other circulating and genetic factors in association to Alzheimer's type dementia. Clin Biochem. 2009, 42: 783-790. (IF = 2. 019).

Diogo L, Grazina M, Garcia P, Rebelo O, Veiga MA, Cuevas J, Vilarinho L, de Almeida IT, Oliveira CR. Pediatric mitochondrial respiratory chain disorders in the Centro region of Portugal. Pediatr Neurol. 2009, 40: 351-356. (IF = 1.497).

Silva MJ, Pinheiro A, Eusébio F, Gaspar A, de Almeida IT, Rivera I. Pyruvate dehydrogenase deficiency: identification of a novel mutation in the PDHA1 gene which responds to amino acid supplementation. Eur J Pediatr. 2009, 168: 17-22. (IF = 1.634).

Master and PhD thesis

Master thesis

Maria Madalena Henriques Serras Vicente Barroso. Caracterização dos efeitos provocados pela acumulação de S-adenosil-homocisteína na expressão dos genes envolvidos na biodisponibilidade de óxido nítrico. Master in Human Molecular Biology, University of Lisbon, Portugal, 2009.

Invited lectures and seminars

Not applicable.

Organization of conferences

Not applicable.

Ongoing projects

Somatic expression of *PDHA2* gene - a long dreamed therapy (PPCDT/SAU-MMO/57052/2004). FCT 06-10, 89.9 K€. PI: I. Rivera

Unraveling the in vitro epigenetic effects of intracellular S-adenosylhomocysteine accumulation on nitric oxide bioavailability (PTDC/SAU-GMG/68714/2006). FCT 07- 10, 74.8 K€. PI: R. Castro.

Production of a stable form of human phenylalnine hydroxylase: towards the 3D structure determination (PTDC/QUI/64023/2006). FCT 09-11, 80.1 K€. PI: P Leandro.

The use of nanobiomaterials for structural and functional protection of human phenylalanine hydroxylase: towards a new approach to phenylketonuria treatment (PTDC/EBB-BIO/101237/2008). FCT 10-12, 124 K€. PI: A.J. Almeida (NanoDDS, iMed.UL).

Erros Hereditários do Metabolismo (Inborn Errors of Metabolism) (008-81–Alto Comissariado da Saúde) 2009-2010, 105 K€. PI: ITAlmeida.

Factores de risco na Doença Cardiovascular (*Risk factors in Vascular Disease*) (M09-49–Alto Comissariado da Saúde), 2009-2012, 150 K€. PI: ITAlmeida.

Genotipagem da população hiperfenilalaninémica portuguesa (*Genetic characterization of the Portuguese Hyperphenylalaninemic population*) (Funded by Merck SA.), 2010, 25K€. PI: I. Rivera.

7.4 Chemical Biology and Toxicology

Objectives

This group is formed by a multidisciplinary team focused on the knowledge of biotransformation, bioactivity, chemical and cellular interactions and mechanistic toxicology, in order to improve key-processes in the context of iMed.UL strategic goal: Drug discovery, Design and Development.

The group aims at improving, characterizing and applying bioactive compounds, as well as studying the mechanisms underlying the toxicity of different xenobiotics, its modulation, identification and validation of predictive biomarkers to be used in prevention of human health risks due to toxic agents.

In this context during 2009 the following objectives have been proposed:

Production of anti-inflammatory compounds by biotechnological processes

The main objectives are the improvement of enzymes activity, selectivity and stability towards the production of compounds with anti-inflammatory activity (e.g. neuroprotective). Innovative enzyme immobilization methods and matrices will be developed and biokinetic studies assessed through special experimental designs and studies on pressure, temperature and pH effects on enzymatic catalysis to modify the reaction mechanism.

New amino acid-based surfactants with different structures (e.g. -amino acid-based surfactants and surfactants derived fromω,α sulfur-containing amino acids) were prepared. The interaction of the surfactants with molecules of biological and pharmaceutical relevance has been evaluated and the mixed systems surfactant-phospholipid, surfactant-protein and surfactant-cyclodextrin studied.

Synthesis, structural characterization and biological evaluation of macrocyclic complexes

Evaluation of the effect of superoxide dismutase mimetic compounds (SODm) on the oxidative injury induced by different oxidants in mammalian cells. The SODm under study are manganese porphyrins as well as two copper(II) macrocyclic complexes, [Cu([15]aneN4O)] and [Cu(ac4[15]aneN4O], selected from our previous studies.

Mechanistic Toxicology, Biomarkers and Risk Assessement

Study of the toxicity of xenobiotics at molecular and cellular levels, research on the interaction of neurotoxicants with chemoprotectors and the selection of adequate toxicogical biomarkers to be used in environmental, occupational, food and clinical safety assessment. Specifically, we aimed to characterize the mechanisms of carcinogenicity of microcystins, as well as the mechanisms of genotoxicity of acrylamide and glycidamide in mammalian cell models. The further study of the nutritional properties of CLA isomers is also an important issue to be pursued in 2009.

Incorporation of emerging antibiotics into orthopaedic acrylic bone cement

This on-going project aims to overcome bacterial resistance with the use of antibiotic-loaded acrylic bone cement in order to reduce the risk of post-operative infection in orthopaedic surgery. A recent broad-spectrum antibiotic -tigecycline - will be incorporated in the polymer; if necessary a second antibiotic – tobramycin – will be added to the bone cement. This project has been developed in collaboration with the nanomedicine and drug delivery system Unit of iMed.UL.

Principal investigator

Matilde da Luz dos Santos Duque da Fonseca e Castro, Full Professor

Research team

António Taco Calado, Full Professor
Maria Camila Batoréu, Associate Professor
Maria Aida Duarte, Associate Professor
Maria Henriques Ribeiro, Associate Professor
Judite Conceição Costa, Assistant Professor
Maria Fátima Cabral, Assistant Professor
Lídia Veloso Pinheiro, Assistant Professor
Ana Francisca Bettencourt, Assistant Professor
António Alfaia, Assistant Professor
Ana Paula Marreilha Santos, Assistant Professor
Maria Luísa Mateus, Assistant Professor
Cristina Carvalho, Assistant Professor
Nuno Filipe Oliveira, Assistant Professor
Célia Faustino, Assistant Professor
Joana Miranda, Assistant Investigator (Ciência 2008 Program)
Ana Sofia Fernandes, PhD student FCT
Carla Tecelão, PhD student FCT
Dinamene Santos, PhD student FCT
Helder Vila-Real, PhD student FCT
Mário Nunes, PhD student FCT
Mário Correia, PhD student FCT
Vasco Branco, PhD student FCT
Ana Cristina Matos, PhD student FCT
Cláudia Silva, PhD student FCT
Isabel Monge, PhD student FCT
Lino Mendes, PhD student FCT
Maria Cristina Neves, PhD student FCT
Maria Edite Torres, PhD student FCT
Sofia Caria, PhD student FCT
Ana Narciso, Master student
Cátia Pereira, Master student
Nuno Félix, Master student
Nuno Torres, Master student
Rita Moura, Master student
Rui Lucas, Master student
Sofia Cerqueira, Master student
Sofia Teixeira, Master student

Main achievements

Production of anti-inflammatory compounds by biotechnological processes

In line with our data on the anti-inflammatory activity of the glycoside, naringin and its aglycone, naringenin, we further showed, in an acute model of induced colitis in mice, that the treatment with both compounds significantly reduced the formation of intestine edema, suggesting an important anti-inflammatory activity (Amaro et al.,2009). To produce interesting bioactive compounds the activity, stability and biokinetics of the biocatalyst were optimized. Naringinase immobilized in different matrices were used to deglycosylate naringin towards naringenin. Microstructured non-ionic polymeric materials XAD-16N and XAD-1600 were suitable for adsorption/covalent immobilization of naringinase (Amaro et al. EFR 2009), as well as polyvinyl-alcohol (PVA) hydrogels (Nunes et al.,

accepted 2009). In addition, the developed PVA-alginate matrices evidenced physical stability at high temperatures, an important achievement due to the reduction of the viscosity of the bioconversion medium, reduction of the risk contamination and improved naringin solubility (Nunes et al., accepted 2009). The production of extracellular polygalacturonase using cells immobilized in calcium alginate was rather innovative and promising with the potential of high productivity (Gattás et al. EFRT 2009). Additionally, central composite design and response surface methodology were used to optimize processes of enzymatic hydrolysis (Ferreira et al., 2009, Amaro et al. 2009). High pressure showed to be a powerful tool to increase enzyme (naringinase) activity and stability against thermal denaturation (Ribeiro et al., accepted 2009). One book chapter on enzymatic deglycosylation systems with applications on pharmaceuticals were published (Ribeiro MH. , 2009).

Finally, new anionic urea-based surfactants derived from □,□-amino acids were synthesized and their solution properties were characterized by electrical conductivity, equilibrium surface tension and steady-state fluorescence spectroscopy techniques (Faustino et al., 2009). Regarding to the gemini surfactant-protein interactions, the effects of pH, temperature, and surfactant stereochemistry were studied (Faustino et al. 2009). These developed surfactants presented anti-microbial activity.

Synthesis, structural characterization and biological evaluation of macrocyclic complexes

Manganese porphyrins are very promising catalytical superoxide dismutase mimics (SODm). MnTM-4-PyP, a member of this class, showed to be a strong protective agent against the toxicity of tert-butylhydroperoxide (an analogue of lipid hydroperoxides) and xanthine/xanthine oxidase system (an extracellular superoxide anion generator), markedly increasing the cell viability and reducing the intracellular level of ROS (Fernandes et al., accepted 2009).

Mechanistic Toxicology, Biomarkers and Risk Assessement

The mechanisms underlying microcystin-LR (MCLR) tumour promotion was studied in a kidney Vero E6 cell line after demonstrating that this is the most sensitive cell model to study the nephrotoxicity of MCLR (Dias et al., 2009). Ultraestrutural analysis showed that the primary target of MCLR is the endoplasmatic reticulum (ER) and that autophagy, apoptosis and necrosis are induced in a dose and time dependent manner(Alverca et al., 2009).

Studies focusing on acrylamide (AA), a suspected human carcinogen were also carried out, using glutathione (GSH) status modulators. Buthionine sulfoximine (BSO, GSH synthesis inhibitor) pre-treatment increased the cytotoxicity and the frequency of aberrant cells excluding gaps (ACEG) induced by AA. While the pre-treatment with GSH-monoethyl ester did not modify cytotoxicity or the frequency of ACEG induced by AA, the co-treatment of AA with GSH decreased both parameters, protecting the cells. Overall, these results reinforce the role of GSH in the modulation of the cytotoxic and clastogenic effects induced by AA (Oliveira et al., 2009).

Conjugated linoleic acid isomers (CLA) is one group of fatty acids with an impressive range of promising health benefits, which can be regarded as useful tools to mitigate cellular toxic events. CLA is present in fat meat of ruminants and in the milk and derived products. Our results showed that intramuscular fat of four Portuguese bovine meats produced is of greater nutritional quality (due to higher n-3 PUFA and CLA content) when compared to intensively produced beef from crossbred young bulls (Alfaia et al., 2009). The effect of pasture intake in CLA contents and its isomeric distribution in milk, cheese and butter from Azores, a Portuguese archipelago in the Atlantic Ocean were also carried out. Butter was the richest product in CLA, whereas cheese presented the highest specific CLA content. The Azorean dairy products have a relatively high amount of CLA, especially the

cis-9,trans-11 isomer, constituting therefore a good source of these compounds for the human diet (Pestana et al., 2009).

We also investigated the combined effects of CLA and dietary fat from distinct sources, animal and vegetable, on the serum adipokine profile of obese Zucker rats. In addition, the fatty acid composition of epididymal and retroperitoneal adipose tissues was determined and a principal component analysis (PCA) was used to assess possible relationships between fatty acids and serum metabolites. Atherogenic diets (2 % cholesterol) were formulated with palm oil and (ovine fat and supplemented or not with 1 % of a mixture (1:1) of cis-9,trans-11 and trans-10,cis-12-CLA isomers. Summing up, CLA added to vegetable saturated enriched diets, relative to those from animal origin, seems to improve the serum profile of adipokines and inflammatory markers in obese Zucker rats due to a more favourable fatty acid composition (Martins et al., 2009).

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Master and PhD thesis

PhD thesis

Cláudia Afonso. Produtos de pesca capturados na costa portuguesa – Benefícios e perigos associados ao seu consumo. University of Lisbon, Portugal, 2009.

Cristina Maria Riscado Mateus Alfaia. Contribution to the studyt of lipid composition and nutritional value of intramuscular fat in ruminant meats. Technical University of Lisbon, Portugal, 2009.

Célia Maria Cardona Faustino. Study of new amino acid-based surfactants. University of Lisbon, Portugal, 2009.

Elsa Maria Alves Dias. Assessment of carcinogenic potential of microcystins. University of Lisbon, Portugal, 2009.

Invited lectures and seminars

Duarte, A. Factores de virulência bacteriana e antibióticos na infecção urinária. Conference in Actualidades sobre a abordagem de infecções Urinárias de Repetição, VI Congresso Nacional da Associação Portuguesa de Neuro-Urologia e Uro-Ginecologia (APNUG), Aveiro, Portugal, 2009.

Duarte, A. Infecções urinárias adquiridas na comunidade. Fosfomicina trometamol na terapêutica das infecções urinárias – *Conference in* Prevalência de bactérias isoladas em mulheres com cistites não complicadas , Porto, Figueira da Foz e Lisboa, Portugal, 2009.

Organization of conferences

Not applicable.

Ongoing projects

Biomarkers of dietary acrylamide exposure: human toxicological relevance PTDC/SAU-OSM/105572/2008; PI: Jorge Gaspar (FCM-UNL), Team member iMED-CBT: Nuno Oliveira (nuclear CV), Matilde Castro, Joana Miranda. FFUL 69.4 K€. (01-02-2010 / 31-01-2013);

PROFLUX - Processes and Fluxes of Mercury and Methylmercury in a Contaminated Coastal Ecosystem (Tagus Estuary, Portugal), PTDC/MAR/102748/2008, FCT, MCTES (2009-2013); PI: João Canário (IPIMAR), Team member iMED-CBT: Cristina Carvalho (nuclear CV) M.Camila Batoréu . FFUL 43.4 K€. (01-03-2010 / 28-02-2013);

Fungi Watch: Benefits and hurdles associated with the presence of fungi in drinking water sources, (PTDC/AAC-AMB/108303/2008). 4 K€.(01-02-2010 / 31-01-2013).

7.5 Medicinal Chemistry

Objectives

The aim of MedChem Group is to contribute to the development of novel lead-drug candidates and molecular diagnostics tools using a pluridisciplinary approach involving (i) validation, overexpression and purification of a biological target (ii) molecular modelling with the biological target in order to rationally design molecules capable of generating the desired biological response, (iii) isolation of novel chemical scaffolds from natural sources that display relevant biological activity, and (iv) synthesis of the selected molecules using selective and efficient strategies. For 2009 the goals included:

Neurodegenerative disease-modifying agents.

Design new compounds that (i) prevent the aggregation of Aβ by inhibiting the PAS of AChE; (ii) cause a modest inhibition of AChE catalytic site, to enhance cholinergic neurotransmission and minimize the peripheral adverse effects of stronger inhibitors; (iii) prevent the depletion of monoamine neurotransmitters, minimize ROS production and apoptotic neuronal death, by inhibiting the MAO enzymes.

Rational design and synthesis of potent inhibitors directed for the major caspase targets that play key roles in apoptotic cell death associated to excessive neuronal apoptosis. Novel scaffolds acting as irreversible inhibitors or transition-state analogues will be designed using in silico structure-based drug design approaches.

Anticancer and anti-inflammatory agents

Discovery and lead optimization of effective anticancer compounds from Euphorbia Tabernaemontana and Plecthrantus species by (i) computer-aided optimization of the natural scaffolds into selective Pgp inhibitors, (ii) search for novel antineoplastic agents, with emphasis on targeting MDR cancer cells, and investigation of their mechanisms of action, and (iii) structural modifications of isolated lead compounds.

Based on the hypothesis that rate of serine acylation by acylating agents is controlled by the molecular recognition by the protein as well as by the intrinsic chemical reactivity, the objective is to design and synthesize novel mechanism-based inhibitors for neutrophil serine proteases involved in inflammatory pathologies as COPD. Scaffolds will be selected by in silico studies which analyze the interactions between the ligands and enzyme active site.

Novel antimalarial and antitubercolous agents

To probe novel chemical approaches to antimalarial combination therapy, by (i) investigating bifunctional molecules designed to kill resistant P. falciparum parasites and simultaneously modulate the emergence of resistance to the compound, and (ii) combining semi-synthetic or synthetic peroxide-type scaffolds with inhibitors of cysteine proteases from P. falciparum in order to delay or overcome the selection of resistance.

To discover inhibitors of the mitochondrial electron transport chain from *P. falciparum*, specifically compounds that target cytochrome b, a critical element of the respiratory complex III, also known as bc1 complex.

To develop pyrazinamide prodrugs as potential antitubercolous agents designed to be incorporated in liposomes and to release the parent drug quantitatively through selective activation by pyrazinamidase from *M. tuberculosis*.

Novel synthetic methods for the preparation of bioactive compounds~

Development of synthetic organic methodologies (i) based on a new concept wherein the expected high levels of reactivity and selectivity stem from a cooperative effect between a metal and an organo catalyst and (ii) relying on starting material from a chiral pool. This innovative approach explores the unique reactivity of metal complexes with the ability of organo-catalysts to induce high levels of enantioselectivity.

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Main Achievements

Neurodegenerative disease-modifying agents.

Tacripyrines were found to be selective and potent AChE inhibitors in the nanomolar range and one compound moderately inhibited the proaggregating action of AChE on the Aβ and the Aβ self-aggregation. Tacripyrines are neuroprotective agents, show moderate Ca2+channel blocking effect, and cross the blood−brain barrier, emerging as lead candidates for treating AD (JMC 2009, 2724).

Naphtho[2,3-d]isoxazole-4,9-diones were found to exert a potent protective role in camptothecin-induced apoptosis in primary rat hepatocytes, by significantly increasing cell viability, while reducing nuclear fragmentation, caspase-3, -8 and -9 activation, and cytochrome c release induced by camptothecin (CBI 2009, 175).

Anticancer and anti-inflammatory agents

Three novel beta-carboline indole alkaloids were isolated from the methanol extract of the leaves of Tabernaemontana elegans (JNP 2009, 1147). In addition, a novel monoterpene indole alkaloid was also isolated from methanol extract of leaves of Tabernaemontana elegans, showing significant apoptosis induction activity in human hepatoma HuH-7 cells (BMCL 2009, 4255).

Coleon U, a diterpene isolated from Plectranthus grandidentatus and with an antiproliferative effect on several human cancer cell lines, was found to selectively induce an apoptotic pathway dependent on nPKC-delta and -epsilon activation, thus representing a promising compound for evaluation as an anti-cancer agent (BP 2009, 449).

Novel cucurbitacins, balsaminagenin A and B and balsaminoside A and the know cucurbitacin karavelagenin C, isolated from the aerial parts of Momordica balsamina L., together with five new mono or diacylated derivatives of karavelagenin C were found to exhibit a strong MDR reversing activity on human MDR1 gene transfected mouse lymphoma cells, comparable to that of the positive control, verapamil. Moreover, the interaction between doxorubicin and these compounds synergistically enhanced the effect of the anticancer drug (BMC 2009, 6942). A curcubitane-type triterpene isolated from Momordica balsamina L. (JNP 2009, 2009) was found to inhibit the efflux of ethidium bromide (EB) on a real-time basis from the ABCB1-transfected mouse lymphoma cell (AR 2009, 3989).

A more detailed understanding of the mechanism behind the powerful action of hypericin, arising as a result of light excitation was achieved using molecular dynamics simulations in a dipalmitoylphosphatidylcholine lipid membrane. (JCTC 2009, 3139).

Antimalarial, antituberculosis and other anti-infectious agents

Artemisinin–vinyl sulfone hybrid molecules were found to be active against the P. falciparum W2 strain in the low nanomolar range and those containing the Leu-hPhe core inhibited falcipain-2 in low micromolar range (BMCL 2009, 3229).

Pyridon-4-imines containing lipophilic side chains at the imine nitrogen atom were found to display antiplasmodial activity against the P. falciparum W2 (chloroquine-resistant) and FCR3 (atovaquone-resistant) strains. Molecular modelling studies suggest that pyridon-4-imines may bind to the ubiquinol oxidation Qo site of cytochrome bc1 (BMCL 2009, 3476)

Imidazolidin-4-one derivatives of primaquine (imidazoquines) were found to display potent in vivo transmission-blocking activity, in vitro tissue-schizontocidal activity, and in vitro anti-Pneumocystis carinii activity. Imidazoquines' stability against both oxidative deamination and proteolytic degradation suggest that they will probably have higher oral bioavailability and lower hematotoxicity than primaquine (JMC 2009, 2506).

Lipophilic esters of pyrazinoic acid (POA), the active metabolite of the antituberculosis agent pyrazinamide (PZA) were found to be active in concentrations 10-fold lower than those needed for PZA to kill sensitive M. tuberculosis and also have a suitable stability in the presence of plasma. Lipophilic amides of POA although more stable in plasma displayed lower activity (EJPS 2009, 257).

Novel synthetic methods for the preparation of bioactive compounds

Water was used as the solvent in the Petasis borono-Mannich reaction. With the use of salicylaldehyde, secondary amines and boronic acids, several alkylaminophenols were obtained in considerably high yields in water. By using the same methodology, 2H-chromenes were prepared with the use of vinyl boronic acids (EJOC 2009, 1859)

The enantioselective construction of the 3-ethylindolo[2,3-a]quinolizidine moiety present in numerous indole alkaloids was reported, the key steps being a stereoselective cyclocondensation of (S)-tryptophanol with an appropriate racemic δ-oxoester and a regio- and stereoselective cyclization of the resulting oxazolopiperidones on the lactam carbonyl group (JOC 2009, 1205).

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Master and PhD thesis

Master thesis

Monteiro A.S. Pró-fármacos de triazenos anti-tumorais para estudos ADEPT e MDEPT, Faculty of Sciences, University of Lisbon, Portugal, 2009.

PhD Thesis

Jalmira M. Irreversible Inhibitors of Serine Proteases Based on the beta-Lactam Scaffold as Potential Drug Candidates, Faculty of Pharmacy, University of Lisbon, Portugal, 2009.

Invited lectures and seminars

Not applicable.

Organization of conferences

BioEM09: International meeting on Biomedical Electron Microscopy Applications, IBILI, Coimbra, 04-05 December, 2009; http://www.ff.ul.pt/paginas/jvitor/BIOEM09/Bioem09/Welcome.html

A Collaborative Solution on Tropical Diseases: A Triennial Initiative in Partnership with NIAID/NIH, FLAD, Lisbon, 08-10 July 2009; http://www.fladtropicaldiseases.com/

Ongoing Projects

Design, synthesis and biological assessment of multifunctional compounds as anti-Alzheimer drugs. PTDC/SAUNEU/ 64151/2006. 107.1 K€ (FCT, 2007-2010); PI Maria Carmo Carreiras.

Chemical reactivity as a tool in drug design: modification of the beta-lactam scaffold to improve serine protease inhibition. PTDC/QUI/64056/2006. 68.6 K€ (FCT, 2008-2010); PI Rui Moreira.

Rhodium catalysed C-H bond activation and C-C bond formation in water and bio-compatible solvents - An expeditious way to prepare biologically active heterocycles. PTDC/QUI/66695/2006. 89.5 K€ (FCT, 2008-2010); PI Pedro Góis.

Genomic restriction endonucleases cloning. 35K $USD (New England Biolabs, Inc., USA, 2010 only); PI Jorge Vitor.

Diterpenes as lead compounds for overcoming multidrug resistance in cancer cells: P-glycoprotein modulators and antiproliferative agents. PTDC/QUI-QUI/099815/2008. 152.4 K€ (FCT, 2010-2012); PI Maria José Ferreira

Targeting the mitochondrial electron transport chain of malaria parasites: computer-assisted design & synthesis of bc1 complex inhibitors as antimalarial agents. PTDC/SAU-FCT/098734/2008. 141.6 K€ (FCT, 2010-2012); PI Rui Moreira

Please MOC it! – Metal-Organo-Catalysis an emerging concept. PTDC/QUI-QUI/099389/2008; 99.3 K€ (FCT, 2010-2012); PI Pedro Góis.

A new life for old antimycobacterial drugs: development of prodrugs of pyrazinoic acid activated by mycobacterial esterases as a way to circumvent resistance to pyrazinamide. PTDC/QUI-QUI/099389/2008; 192.9 K€ (FCT 2010-2012); Luís Constantino.

Exploiting the type II phosphomannose isomerase BceAJ as a new target for the development of new antimicrobials and for biotechnological applications. PTDC/EBB-BIO/098352/2008. 6.6 K€ (FCT, 2010-2012); MedChem team: Rita Guedes, Daniel Santos

Recycling antimalarials: rational design of novel 8-aminoquinoline analogues with gametocytocidal and blood schizontocidal. PTDC/QUI/65142/2006. 6.4 K€ (FCT, 2010-2012); MedChem team: R Moreira.

7.6 Nanomedicine and Drug Delivery Systems

Objectives

The main overall research objectives currently defined for the area of Nanomedicine and Drug Delivery Systems:

(i) achieving intracellular delivery of nucleic acids and/or combination chemotherapy by the use of nano-systems for cell (cytosolic) specific delivery in oncology and inflammation

(ii) attaining effective mucosal vaccination through micro and nano-systems, as well as design of well suited therapeutic systems for intracellular pathogens

(iii) developing mechanistic and technological innovative approaches for dermal delivery in different conditions such as inflammation, cancer and/or autoimmune diseases

(iv) using innovative approaches using macromolecular complexation and particle engineering for pulmonary delivery, as well as for other non-parenteral routes.

Principal investigator

Rogério Paulo Pinto de Sá Gaspar, Full Professor

Research team

Maria Eugénia Meirinhos Cruz, Principal Investigator
António José Leitão Neves Almeida, Associate Professor
Helena Maria Cabral Marques, Associate Professor
Maria Bárbara dos Anjos Figueira Martins, Principal Investigator
João Fernandes Abreu Pinto, Associate Professor
Maria Luísa Teixeira de Azevedo Rodrigues Corvo, Assistant Investigator
Helena Margarida de Oliveira Marques Ribeiro, Assistant Professor
Maria Manuela de Jesus Guilherme Gaspar, Assistant Investigator
Sandra Isabel Dias Simões, Assistant Investigator
Mafalda de Castro Ascensão Marques Videira, Assistant Professor
Helena Isabel Fialho Florindo, Assistant Professor
Paulo José Pinto Salústio, Assistant Professor
Lidia Maria Diogo Gonçalves, Assistant Investigator (Ciência 2007 Program),
Liana Casquinha da Silva, PhD, Assistant Investigator (Ciência 2008 Program)
Patricia Ferreira da Luz Pereira, Postdoc FCT
Lara Carolina Alvarez de Lacerda, Postdoc FCT
Maryia Brachkova, PhD student FCT
Manuela Colla Carvalheiro, PhD student
Marta Cristina Jorge Cabral Machado, PhD student
Gonçalo Emanuel Rodrigues da Cunha Correia de Oliveira, PhD student
Andreia Patrícia Henriques Ascenso, PhD student (Univ. Assistant)
Sara Sofia Caliço Raposo, PhD student
Rui Manuel Jesus Lopes, PhD student
Joana Catarina Mendão Azeitão da Silva, PhD student
Giuliana Mancini, PhD student
Paulo Roque Lino, PhD student
Ana Cristina Matos, PhD student
Pedro Miguel Mendes Martins, junior investigator
Ana Cristina Cadete Pires, junior investigator
Monique Toledo, junior investigator
Ana Salgado, Pharmacist, Technician

Carla Vânia Pereira Eleutério, Technician
Isabel dos Prazeres Costa Marques Mascarenhas Ataíde, Technician
Maria de Lourdes Machado Teixeira, Administrative support

Main achievements

The group main achievements can be structured according to the four main research areas/objectives of the Nanomedicine & Drug Delivery Systems group.

Oncology and inflammation

Initial stages of project focused in targeting with combination therapy for breast carcinoma and leukemia, through the development of new innovative materials (Iberian Technology Platform coordinated by CNC, in collaboration with Institut Principe Filipe, Valencia, Spain - Advances in drug delivery: targeted combined therapy for breast carcinoma and leukemia, OncoTargetNanoMed, Nano/Nmed-AT/0042/2007) centered in solving major pitfalls of translational research with such systems (Gaspar and Duncan, 2009).

Two separate projects looking at targeting to cancer stem cells in breast cancer. The first one in its initial stage through an European collaboration (Targeting Combined Therapy to Cancer Stem Cells - NanoStem, ENMED/Nmed/0001/2009, funded through EuroNanoMedNet a Nanomedicine ERA-NET) and the second pursuing a collaboration with Alliance for Nano-Health and MD Anderson in Houston, Texas in order to achieve the construction of a multistage delivery system (Klopp et al, submitted).

Mechanistic characterization of lipid rafts associated with sphingomyelinase activity and ceramide-induced membrane physical alterations (of critical importance for cytosolic delivery strategies) (Silva et al, 2009)

Infectious Diseases

Synthesis of new derivatives of dinitroanilines and demonstration of superiority of their liposomal formulations and of paramomycin as compared to the free compounds (>90% parasite load inhibition) (Carvalheiro et al., 2009).

Construction of recombinant DNA vaccine, in vivo and in vitro assessment of NP vaccine. Characterisation of new protease inhibitors and development of polymeric nanoparticulate vaccine for intracellular delivery of DNA (Rodrigues et al, 2009)

Rifabutin and paramomicin liposomal formulations with enhanced antimycobacterial activity (Figueiredo et al, 2009). .

S. equi antigens successfully associated to polymeric nanospheres; Ab and cytokine titers predict protection (new nanoparticulate carriers being used) (Florindo et al, 2009).

Demonstration of in vitro activity of new extracts from medicinal plants against MRSA strains.

Dermal delivery

Identification of precise mechanisms of action upon dermal delivery, using a combination of appropriate biophysical techniques, mathematical modelling and chemometric analysis (Santos et al, 2009).

Development of new formulations with anti-inflammatory drugs and chemical enhancers. Improvement of drug stability using nanosystems (SLN, Liposomes, CyD complexes). Establishment of innovative technological approaches for emulsification processes. Development of deformable lipid vesicles for local and systemic inflammatory desorders (Simões et al, 2009).

Macromolecular complexation & Particle engineering

Understanding the best spray-drying conditions for drug-cyclodextrins complex formation leading to particles engineered for lung delivery. Supercritical CO2 as a new method for drug-cyclodextrins complex formation (Cabral-Marques and Almeida, 2009).

Understanding the effect of the amount of solvent on the drug-cyclodextrins complex formation by kneading method (Salústio et al., 2009).

Use of liposomal systems for pulmonary delivery. (v) Construction of a computational model describing the flow of airborne particles in tubes with complex geometry (Mendes et al, 2009).

Production of pellets by extrusion and spheronisation based on modified hidroxyapatite, and new application of extrusion and co-extrusion technology on the design of new dosage forms (laminar extrusion).

Evaluation of the emulsification kinetics of self-emulsifying systems (Santhagunam Aruna, MSc Thesis, 2009).

Bacterial immobilization (e.g. Lactobacilli) in granules and pellets (Brachkova et al, 2009).

Publications

Ascenso A, Marques H C. Acne in the Adult. Mini-Rev Med Chem. 2009, 9: 1-10. (IF = 2.971).

Brachkova MI, Duarte A, Pinto JF. Evaluation of the viability of Lactobacillus spp. after the production of different solid dosage forms. J Pharm Sciences. 2009, 98: 3329-3339.(IF= 2.906).

Cabral-Marques H, Almeida R. Optimisation of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes Eur J Pharm Biopharm. 2009. 73: 121–129. (IF= 3.151)

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Florindo HF, Pandit S, Gonçalves LMD, Alpar HO, Almeida AJ. New approach on the development of a mucosal vaccine against strangles: systemic and mucosal immune responses in a mouse model. Vaccine, 2009, 27: 1230-1241. (IF = 3.616).

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Gaspar R, Duncan R. Polymeric carriers: Preclinical safety and the regulatory implications for design and development of polymer therapeutics. Adv Drug Deliver Rev. 2009, 61: 1220–1231. (IF = 11.957).
Gaspar R. The regulatory landscape: implications for design and development of nanomedicines. J Pharm Pharmacol. 2009, 61: A148-A149 (IF = 1.742).
Kreiner M, Chillakuri CR, Pereira P, Fairhead M, Li ZH, Mardon HJ, Holt SA, van der Walle CF. Orientation and surface coverage of adsorbed fibronectin cell binding domains and bound integrin α5β1 receptors. Soft Matter. 2009, 5: 3954-3962. (IF = 4.869).
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Mendes PJ, Sousa JMM, Pinto JF. Prediction of the deposition of dry powder aerosols. AAPS J. 2009, 11: 186-194. (IF = 5.540).
Rodrigues MA, Li J, Padrela L, Almeida A, Matos HA, de Azevedo EG. J Anti-solvent effect in the production of lysozyme nanoparticles by supercritical fluid-assisted atomization processes. J Supercrit Fluid. 2009, 48: 253-260. (IF = 2.639).
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Santos P, Machado M, Watkinson AC, Hadgraft J, Lane ME. The effect of drug concentration on solvent activity in silicone membranes. Int J Pharmaceut. 2009, 377: 70-75. (IF = 2.962).
Silva LC, Futerman AH, Prieto M. Lipid raft composition modulates sphingomyelinase activity and ceramide-induced membrane physical alterations. Biophys J. 2009, 96: 3210-3222. (IF = 4.390).
Simões S, Marques C, Cruz ME, Martins MBF. Anti-inflammatory effects of locally applied enzyme-loaded ultradeformable vesicles on an acute cutaneous model. J Microencapsul. 2009, 26: 649-658. (IF = 1.890).

Master and PhD thesis

PhD thesis

Paulo José Salústio. Promotion of increased solubility of poorly soluble drugs for oral administration dosage forms of controlled or immediate release" (Promoção do aumento da solubilidade de fármacos pouco solúveis para a sua administração oral em formas farmacêuticas de libertação imediata ou controlada). University of Lisbon, Portugal, 2009.

Master thesis

Teresa Calejo. Colagénio de Catostylus tagi como matriz polimérica destinada à veiculação de fármacos proteicos, 2009.

Santhagunam Aruna. Evaluation of emulsification kinetics of oil in water, 2009.

Margarida Ramos. Complexos Omeprazol-Ciclodextrina: Estudos de Estabilidade de Actividade Bioquímica, 2009.

Invited lectures and seminars

Cruz, MEM. Nanoformulations of Trifluralin Analogues as Antileishmanial Agents, Expert Meeting of the COST Action CM0801, Blagoevgrad, Bulgaria, 2009.

Gaspar, R. Regulation of nanopharmaceuticals and devices, ESF/UB, 2nd European Summer School in Nanomedicine, Cascais, Portugal, 2009.

Gaspar, R. Regulatory issues: implications for design and development of nanomedicines, 1st International Summer School Nanomaterials and Nanotechnologies in Living Systems, Moscow State University and RUSNANO, Zaria, Russia, 2009.

Gaspar, R. Impact of regulations on biopharmaceutical R&D : an European perspective and the need to improve the development of new pharmaceuticals and biosimilars, Biopharmaceutical process development and regulatory issues, UFRJ-COPPE, Rio de Janeiro, Brazil, 2009.

Gaspar, R. Regulatory issues: implications for design & development, The British Pharmaceutical Conference 2009, Manchester Central, United Kingdom, September, 2009.

Gaspar, R. Nanotechnology: risk governance looking at medicines / nanopharmaceuticals regulation as an example, Roundtable on Risk Governance Policy for Nanotechnology (OECD WP Nanotechnology), Vienna, Austria, 2009.

Gaspar, R. Scientific Issues and Regulation Aspects in the Translation of Nanopharmaceuticals to Clinic, NanoDDS 2009: From Laboratory to Clinical Reality, Indianapolis, Indiana, USA, 2009.

Gaspar, R. Impact of regulations on biopharmaceutical R&D: an European perspective and the need to improve the development of new pharmaceuticals and biosimilars, 1st Forum on Biotech Products, S. Paulo. Brazil, 2009.

Gaspar, R. Nanomedicine: 30 years of clinical experience, lessons learned and trends for translational research, Workshop Nano09, INL, Braga, Portugal, 2009.

Almeida AJ, Salgado AC. Desenvolvimento e controlo de qualidade de medicamentos manipulados. Simpósio Novos Medicamentos Manipulados e Novos Sistemas Terapêuticos. Universidade Fernando Pessoa, Porto, Portugal, 2009.

Gaspar, R. What is Nanomedicine? From lab to clinic. Nanomedicine Nanocourse , IMM, Lisbon, Portugal, 2009.

Cruz, MEM, Drug Delivery Systems: the example of Liposomes, Nanomedicine Nanocourse, IMM, Lisbon, Portugal, 2009.

Gaspar, R. Nanomedicine: converging sciences and technologies, the case for nanotechnology in health care, Workshop in Biomedical Engineering (WBME 2009), FCUL, Lisbon, Portugal, 2009.

Almeida AJ. Qualified person: formação e competências. Conferência proferida na Reunião Anual do Colégio de Indústria Farmacêutica da Ordem dos Farmacêuticos, subordinada ao tema Qualified Person: Mito e Realidade, Tróia, Portugal, 2009.

Organization of conferences

The major event organized in 2009 was the 2nd European Summer School in Nanomedicine (European Science Foundation) in which Rogério Gaspar acted as European Director (after having been co-Director of the first School held in 2007 in Cardiff, UK), and several members of the group served as local organising committee.

From the report submitted to the European Science Foundation (ESF):

The meeting was heavily oversubscribed and this necessitated increasing the intake to 210 delegates (including ~ 40 faculty and guest lecturers).

The meeting program comprised 3 Plenary Lectures (experts from USA-Japan-Europe) to give a global, truly leading edge perspective, 22 expert lectures (these world renown speakers were asked to introduce their field at undergraduate level and progress to specific research level examples) and 48 short presentations from the poster communications chosen to complement each of the core sessions with real ongoing research examples. There were 105 posters and they were by necessity split into 2 sessions. Optional tutorials and discussion groups were offered in the afternoons (>12) and the delegates selected the topics for these.

Delegates came from ~40 countries, mostly from Europe, but also the USA, Argentina, Japan, Singapore, South America, Africa and Australia. Importantly for the first time there were delegates from the Middle East e.g. Iran, Iraq, Saudi Arabia, Palestine.

The perceived need for such integrated training (laboratory to clinic) of converging sciences also helped to attract considerable additional sponsorship from companies (Merck, GSK, Novartis Alliance Healthcare, Schering Plough, Medinfar) academic institutions (iMed.UL of the Faculty of Pharmacy University of Lisbon, Cardiff University, Zaragoza ICMA, CDDN University of Nebraska, University of Utrecht, CICECO University of Aveiro), national funding bodies (EPSRC Platform in Nanomedicine) and national academies (Academy of Pharmaceutical Science and Technology of Japan). This support enabled the award of over 73 grants to aid participation of young academic scientists and medical doctors.

The course was supported by > 30 of the world leading experts in Nanomedicine representing all areas of Nanomedicine – from materials science and engineering, analytical tools, to the medical challenges, the regulatory perspective, clinical trial design and societal & ethical aspects of this newly emerging field.

Ongoing projects

2009-2011 - Iberian Nanotechnology Platform, Nano/Nmed-AT/0042/2007 (iMed.UL: € 52.000) (iMed.UL PI: RG) - Advances in drug delivery: targeted combined therapy for breast carcinoma and leukemia (OncoTargetNanoMed)

2010-2013 - ERA-NET Nanomedicine, ENMED/Nmed/0001/2009 (iMed-UL: € 200.000) (iMed.UL PI: RG) - Targeting Combined Therapy to Cancer Stem Cells (NanoStem)

Contract-research: ONCOTERA – Discovery and development of new compounds for colon cancer therapy (QREN, ECBIO) (€ 83.795) (iMed.UL PI MEMC)

2010-2012 - PTDC/CVT/098290/2008 (PI: MEMC) (iMed.UL: €124.610) - A challenge for the treatment of parasitic diseases: rational design of trifluralin derivatives and appropriate nanoformulations

2010-2012 - PTDC/EBB-BIO/101237/2008 (PI: AJA) (iMed.UL: €124.000) - The use of nanobiomaterials for structural and functional protection of human phenylalanine hydroxilase: towards a new approach to phenylketonuria treatment

2010-2012 – PTDC/SAU-FCF/101950/2008 (Nano&DDS/iMed.UL: €25.000) (MEMC) - A new life for old antimycobacterial drugs: development of prodrugs of pyrazinoic acid activated by mycobacterial esterases as a way to circumvent resistance to pyrazinamide

2010-2012 - PTDC/EQU-EPR/099226/2008 (iMed.UL: € 20.900) (MEMC) - Development of new polymer-liposome complexes combining experimental and computational approaches

2010-2012 - PTDC/EQU-EQU/104318/2008 (iMed.UL:€ 8.250) (AJA)

Research contract with industry (EDOL) and Univ./Industry grant.

2010-2012 - PTDC/SAU-FCF/098733/2008 (PI: HCM) (iMed.UL: € 193.271) - Lung CYD-DDS (cyclodextrin-based drug delivery systems) for inhalation

2010-2012 - PTDC/CTM/098688/2008 (PI: JFP) (iMed.UL: € 150.000) - Production of co-extrudates containing drug micro and nano particles polimorphs for oral administration of drugs

2010-2012 - PTDC/EME-MFE/103640/2008 (iMed.UL: € 66.621) (JFP) - Optimization of mono and bi phasic microflows in medical devices for powder inhalation (OPTIMED)
2010-2012 – PTDC/QUI-QUI/098216/2008 (iMed.UL: € 47300) (JFP) - Polimorphism in organic molecular solids: structure and energetics
2009-2012 - PTDC/QUI/66086/2006 (iMed.UL: € 10.224) (HCM) - “Green” production of cyclodextrin-based matrixes using supercritical carbon dioxide

7. 7 Pharmacological Sciences

Objectives

The research held in this group focus on two Pharmacological fields: Basic Pharmacology/Pharmacotoxicology and Pharmacokinetics/Toxicokinetics. The specific objectives for each field of research for 2009 were established as follows:

Basic Pharmacology/Pharmacotoxicology Sub-Group (PD/PT)

The research in this field has mainly been designed to address mechanisms involved in 1) inflammation and 2) smooth muscle contractility. The main objective is to study the cellular cascades associated to smooth muscle contractility and inflammation, using in vitro and in vivo models. The cross talk between adrenergic and serotoninergic pathways has been analyzed in samples of rat vas deferens. Multiple molecules with natural and synthetic origin were investigated in order to explore their preventive or curative potential in different types of inflammation (acute, chronic, localized and systemic).

Pharmacokinetics/Toxicokinetics Sub-Group (PK/TK)

The main research area of this field is focus on modeling and simulation. The objectives for 2009 include: (1) the characterization of the pharmacokinetic / biopharmaceutic properties of new molecular entities based on modeling & simulation using molecular descriptors in physiologically based models for prediction of pharmacokinetic behavior; (2) Population pharmacokinetic models for TDM data including non-linear models for phenytoin; (3) the use of metrics for bioequivalence decision in regulatory evaluation.

Pharmacoepidemiology/toxicoepidemiology sub-group (PE/TE)

This group is aimed to address a) the pattern of pharmaceuticals use in elderly and pediatric populations and in patient populations, in particular those with type 2 diabetes, cardiovascular disease, peptic ulcer, kidney transplanted and mental health disorders and development of networks for post-marketing surveillance; b) Implementation and evaluation of Pharmaceutical Care Programs in community and hospital setting and use of Hospital pharmacy as a tool to access incidence of nosocomial infections and hospital admissions due to adverse drug reactions; c) Investigation on the role of Portuguese Community Pharmacy in Health Primary Care..

Principal investigator

Beatriz Silva Lima, PhD, Full Professor, FFUL

Research team

José Guimarães Morais, PhD, Full Professor, FFUL
José Cabrita, Full Professor
Helder Mota Filipe, PhD, Associate Professor, FFUL
Bruno Sepodes, PhD, Assistant Professor, FFUL
Cristina Almeida, PhD, Assistant Professor, FFUL
Cristina de Mello Sampayo, PhD, Assistant Professor, FFUL
Maria Eduardo Figueira, PhD, Assistant Professor, FFUL
Maria do Rosário Lobato, PhD, Assistant Professor, FFUL
Rui Manuel Amaro Pinto, PhD, Assistant Professor, FFUL
Carla Maria Teixeira Barros Branco Ló, Assistant Professor FFUL
Afonso Miguel das Neves Cavaco, Assistant Professor FFUL

Maria Augusta Mendonça Soares, Assistant Professor FFUL
Ana Paula Mecheiro A. Martins Silvestre Correia, Invited Assistant Professor FFUL
Fernando Fernandez-Llimós, Invited Assistant Professor FFUL
Maria Sofia Oliveira Martins, PhD student
Ana Lúcia Marcelino, PhD student
Ana Sofia de Melo Saião, PhD student
João Paulo Cruz, PhD student
João Rocha, PhD student
Nuno Elvas Silva, PhD student
Margarida Estudante, PhD student
Patrícia Marques, PhD student
Paulo Jorge Alves Paixão, PhD student
Pedro Contreiras Pinto, PhD student
Maria de Fátima Pinela Falcão, PhD student,
Maria Filipa Duarte Ramos Carmona, PhD student,
Artur Mendes Moura, PhD student,
Maria Adriana Pereira Henriques, PhD student FCT (2006 -2010)
Isabel Maria Pires Sebastião Ramalhinho, PhD student FCT
Teresa Margarida M Salgado, PhD student FCT
João Pelicano Romano, PhD student FCT
Mariana Neves, BTI Fellow

Main achievements

Main achievements in 2009 are summarized as follows:

Basic Pharmacology/Pharmacotoxicology Sub-Group (PD/PT):

Focused on studying co-transmission pathways and new mediators involved in contractility, ischemia-reperfusion and other inflammatory injuries. Was demonstrated i) A concentration-dependently decrease of phenyleprine-induced rat vas deferens contractility with Fluoxetine concentrations equal and above 5µM. The response was completely abolished at and above 50µ M Fluoxetine concentrations. At lower Fluoxetine concentrations (M 1µM), similar, or even higher, vas deferens contractile responses were observed. A no effect was observed with lower concentrations (0.001 to 1.0µM) of Mirtazapine, while higher concentrations (10µM) increased the contractile response. The results suggest that drugs involved in the modulation of 5-hydroxytryptamine can modulate the vas deferens contractility through a mechanism where NE also plays an important role. ii) the protective role of LPS preconditioning and simvastatin pre-treatment in an experimental model of burn injury in the rat, and also the protective effect of rosmarinic acid in local and systemic models of inflammation in the rat. Has expected, we also published relevant data on the role of hydrogen sulphide pathway in ischemia-reperfusion injury.

Pharmacokinetics/Toxicokinetics Sub-Group (PK/TK)

Pursued projects on: (1) modelling and simulation (prediction of PK/biopharmaceutic properties drug clearance, blood distribution, permeability, bioavailability) using Artificial Neural Networks and Physiologically Based Models (2 doctoral thesis in course (1 being finalised), 2 papers accepted); (2) Role of transporters in BCS class I and II drugs (1 doctoral thesis in course, 1 project submitted) and (3) Population pharmacokinetic models for TDM data: vancomycin (1 doctoral thesis in course; 1 scholarship obtained); (4) cutaneous gas exchange modelling as marker for Peripheral Vascular Disease (1 doctoral thesis in course).

Pharmacoepidemiology/toxicoepidemiology sub-group (PE/TE)

The pattern of use of anti-diabetic drugs in a national sample of type 2 diabetic patients was studied in community pharmacies showing a similar pattern to that described in other European countries.

A cross sectional survey was carried in order to assess the predictors for hypertension prevalence, treatment and control. Hypertension was associated with high-age, being male, married, obesity, hypercholesterolemia, diabetes, family history of premature CVD and n º of visits to physician. Treatment was positively associated with being female, not married, diabetic, urban area and nº of visits to physician. Hypertension control was positively associated with self-reported adherence, perception of the anti-hypertensive medication effectiveness

Several instruments to assess humanistic outcomes in different patient conditions were cross-culturally translated and validated into Portuguese: Diabetes Quality Of Life Measure; hypertension quality of life questionnaire; Pharmacy Services Questionnaire. All of them achieved a Portuguese version presenting good psychometric properties and high reliability to be used in clinical practice and research.

Cognitive services provision in community pharmacies were studied in different aspects. Facilitators to overcome the previously identified barriers for implementing those cognitive services in community pharmacies were established in a qualitative study performed in an international collaboration including Spain and Australia

Publications

Amaro MI, Rocha J, Vila-Real H, Eduardo-Figueira M, Mota-Filipe H, Sepodes B, Ribeiro MH. Anti-inflammatory activity of naringin and the biosynthesised naringenin by naringinase immobilized in microstructured materials in a model of DSS-induced colitis in mice. Food Res Int. 2009, 42: 1010-1017 (IF = 2.414).

Bignotto L, Rocha J, Sepodes B, Eduardo-Figueira M, Pinto R, Chaud M, de Carvalho J, Moreno H Jr, Mota-Filipe H. Anti-inflammatory effect of lycopene on carrageenan-induced paw edema and hepatic ischemia-reperfusion in the rat. Brit J Nutr. 2009, 102:126-133. (IF = 3.446)

Carvalheiro M, Jorge J, Eleutério C, Pinhal AF, Sousa AC, Morais JG, Cruz MEM. Trifluralin liposomal formulations active against leishmania donovani infections. Eur J Pharm Biopharm. 2009, 71: 292-296. (F = 3.151).

Cordovil R, Gouveia L, Araújo D,Davids K, Gouveia L, Barreiros J, Fernandes O, Serpa S. The influence of instructions and body-scaling as constraints on decision-making processes in team sports. Eur J Sport Sci. 2009, 9: 169-179. (IF = 0.742).

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Master and PhD thesis

PhD thesis

Letícia Bignotto. Avaliação dos efeitos do licopeno nas alterações cardiovasculares funcionais induzidas pela inibição cronica da sintese de oxido nitrico e em modelos de inflamação aguda. 2009. (Orientador: Helder Mota-Filipe).

Maria Augusta Mendonça Soares. - Avaliação da terapêutica potencialmente inapropriada no doente geriátrico. Universidade de Lisboa. 2009. (Orientador: JG Morais; Co-orientador: Fernandez-Llimos F).

Master thesis

Maria João Barreira. Ocorrência da Patulina em Alimentos destinados a Lactentes e Crianças: Optimização do Método de Análise por SPE-HPLC/UV". 2009. Classificação: 19,5 valores. (Cristina Almeida).

João Rocha. Avaliação da actividade anti-inflamatória do ácido rosmarínico e de um extracto de Rosmarinus officinalis. 2009. Classificação: 19 valores. (Orientadores: Helder Mota-Filipe e Beatriz Silva Lima).

Invited lectures and seminars

Silva-Lima, B. Recent Experience in Non Clinical Assessment: Scientific advice and marketing Authorization Applications, EMEA Workshop on SMEs/ London, England, 2009.

Silva-Lima, B. The European Situation & Case Studies, Session: Nonclinical development of Pediatric Drugs, 21st Annual EuroMeeting, DIA, Berlin, Germany, 2009.

Silva-Lima, B. Required Preclinical Studies for the Marketing Authorisation of a New Medicinal Product to be Used in Children, EUDIPHARM - Course on Pediatric Drug Development, Universite paris Descartes, Paris, France, 2009.

Silva-Lima, B. Preclinical Studies to Support Clinical Trials in Special Patient Populations (II); Preclinical Studies with Juvenile Animals. Course DIA/ FFUL/Uniiversity of Basel, Basel, Germany 2009.

Silva-Lima, B. Juvenile animal Studies in the context of PIPs in Europe, 45th annual meeting, DIA, San Diego, EUA, 2009.

Silva-Lima, B. Some, Feelings and Expectations on Carcinogenomics, ILSI-HESI workshop on carcinogenomics, Venice, Italy, 2009.

Silva-Lima, B. ECOPA workshop on Replacement in The Development of New Biological Entities, Peptides, mAbs, Budapest, Hungary, 2009.

Silva-Lima, B. Safety Evaluation of Biopharmaceuticals; Update on S6 after November ICH Meeting , 9th Preclinical Assessors Meeting, Lucerne, Switzerland, 2009.

Silva-Lima, B. An European View on Safety Assessment of Biopharmaceuticals, Eurotox CAC1, Dresden, Germany, 2009.

Morais, JG. The New EMEA Guideline on the Investigation of Bioequivalence. Cost Action B25: Physiologically based pharmaco-toxicokinetics and dynamics, Final Congress, Titania Hotel, Athens, Greece, 2009.

Morais, JG. The New EMEA Guideline on the Investigation of Bioequivalence, From Drug Delivery Systems to Drug Release, Dissolution, IVIVC, BCS, BDDCS, Bioequivalence and Biowaivers, Titania Hotel, Athens, Greece, 2009

Morais, JG. The Regulator's view of a Fixed Dose Combination. Is it Worth the Effort? 2nd PharmSciFair (EUFEPS), Nice, France, 2009.

Morais, JG. Introduction: Classical Equivalence Studies; Scientific basis and Limitations, Workshop on Bioequivalence of Complex Drugs, Leiden, Netherlands, 2009.

Organization of conferences

Members from PD/PT group were involved in the organization of: European Shock Society Congress 2009. 24th-27th September 2009, in Lisbon, Portugal

Members from PK/TK group were involved in the organization of: Simpósio Anual da Associação Europeia de Faculdades de Farmácia, 18 – 20 June, 2009, in Oslo, Norway.

Ongoing projects

Funding Granted:

Plurianual Financial Programe of iMed – 64 261,61 EUROS

2007 - 2010; Funding (total): 22 800 EUROS; PTDC/AGR-ALI/65926/2006: Source: FCT.

2006 - 2010; Funding: 12 000 EUROS per year; SFRH/BD/25959/2005; Source: FCT (Bolseiro: Patricia Marques)

2006 - 2010; Funding: 11 000 EUROS per year; SFRH/BD/31025/2006;Source: FCT (Bolseiro: Margarida Estudante)

2006 - 2010; Funding: 11 000 EUROS per year; SFRH/BD/28545/2006;Source: FCT (Bolseiro: Paulo Paixão)

2010 - 2014; Funding: 11 000 EUROS per year; SFRH/BD/64180/2009;Source: FCT (Bolseiro: João Rocha)

2009 - 2010; Funding (total): 10 000 EUROS; GSK (Bolseiro: Sofia Saião)

Fundação Astra-Zeneca (15.000€)

Funding Requested:

Funding application, 20010-2012 period, from FCT projects:

PTDC/SAU-FAR/114913/2009 (Prediction of Intestinal Transporter-Effects on the Absorption of Metabolized Drugs).