Biomedical Studies

Teresa Pinheiro

The research activities within the Biomedical Studies group make use of focused ion beams techniques as well as molecular biology and biochemistry techniques gathering know-how and expertise on a wide range of disciplines from Biochemistry and Biology to Medicine and Physics. The most relevant applications are relative to the study of human pathologies, by assessing elemental distributions in tissues together with clinic, biochemical and genetic indicators. The work performed is carried out exclusively under research contracts that associate several national and international research centres.

The multidisciplinary characteristic of the joint teams also permit to attract young scientists to carry out their B.Sc., M.Sc., and Ph.D. thesis, which programmes of study are often within the activities of financed projects.

Apart from research activities, technical services are provided to private institutions, mainly on the charac-terisation of raw materials for the pharmaceutical industry.

Presently the instrumental capabilities for elemental and morphology determination using ion beams are becoming fully explored and rapidly overwhelmed by new instrumentation developed in the past decade. Thus, some efforts are being done to diversify the analytical possibilities, such as to explore the combination of nuclear microscopy with other microscopy techniques and to determine molecular probes to assess cell/tissue response. New research projects for the forthcoming triennium and the recently approved FCT projects of the programme for infrastructure modernisation, in particular those that will enable the acquisition of modern tools on the analysis of chemical elements and their species will open novel possibilities to broaden research activities on current issues.

The main achievements of the research developed during 2005 are summarised in the following pages.

Research Team

Researchers (*)

T. PINHEIRO, Aux., Group Leader
L.C. ALVES, Aux. (25%)

Students

P. NAPOLEÃO, Ph.D. student, FCT grant
A. VERÍSSIMO, Graduate student, ITN grant
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I. GUINOTE, Graduate student, IEFP grant

Technical Personnel

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Collaborators

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R. SANTOS, M.D., St. Maria Hospital
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Inflammation and redox status in human diseases

P. Napoleão, A. Veríssimo, M.C. Santos[1], A.M. Viegas-Crespo[2],A. Bugalho de Almeida[3], P. Monteiro[3] A. Barreiros[4], L. Veiga[5], T. Pinheiro

The human pathologies highly prevalent in the western population such as atherosclerosis, with a particular emphasis on coronary artery disease (CAD), and chronic obstructive pulmonary disease (COPD), have been the prime areas of research in the past years. How morphologic, metabolic, inflammatory and redox markers can interact to initiate and propagate disease are the common ground of the research activities.

Atherosclerosis, the main cause of CAD, is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate, propagate, and activate lesions in the arterial tree.

The developed research on atherosclerosis under the Project POCTI /ESP/41008/2001-2005, evolved to the study of CAD, under a Ph.D. thesis programme (SFRH/BD/18822/2004) with the collaboration of the Department of Cardiology of Hospital Sta. Marta (Lisbon). Some of the morphologic, metabolic and inflammatory indicators studied, so far, were narrowly interpreted and others included or combined. Presently, global health status is assessed for all patients and healthy cohorts (morphologic data, and systemic indicators, as lipid profile, haemogram, metabolism of Fe, activity of transferases, C-reactive protein, albumin, among others) enabling to progressively establish and enlarge data basis. Pro-inflammatory cytokines (TNF-a, and interleukins), anti-inflammatory interleukins, soluble cytokine receptors and adhesion molecules (e.g. P-selectin), and indicators of metabolism and redox balance, as nitric oxid (NO), LDL oxidation (ox-LDL) and paroxonase activity, respectively, are parameters included in the study.

The different markers are being assessed longitudinally, in patients with CAD, from acute coronary failure along recovery. The variations observed for some of them are illustrated in Fig.1-a,b.

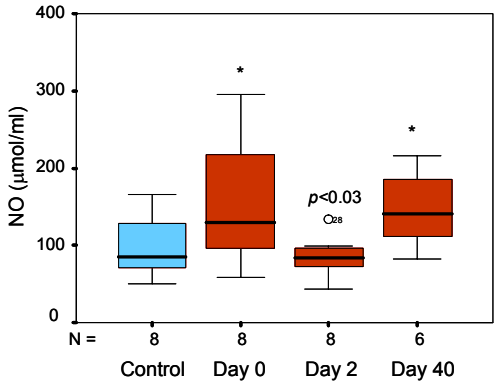

Fig. 1a – NO concentration for controls and CAD patients. Significant differences to controls (*) and within CAD (p-value).

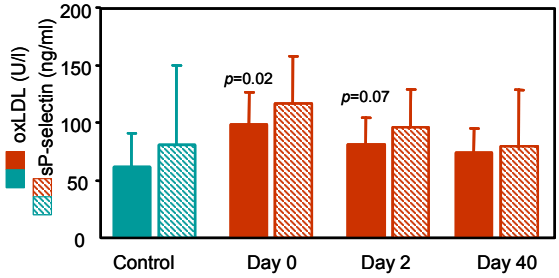

Fig. 1b – Soluble P-seletin and ox-LDL concentrations for controls and CAD patients. Significant differences to controls (p-value).

Chronic Obstructive Pulmonary Disease (COPD) is a highly prevalent disease (affecting ca. 5% of the Portuguese population) associated with airway inflammation. The relationship between functional parameters, and indicators of redox balance and inflammation were studied (Project FCG/SDH.IC.I. 01.22/2001-2005). Changes in inflammatory and redox markers both locally (sputum) and systemic were found for stable and exacerbated patients with COPD. These changes could not be associated to the smoking history and/or habits of the patients, although some of them can be associated to the severity of the disease as is the case for interleukin 8, eosinophilic cationic protein, plasma contents of Fe, and Se, and activities of specific enzymes of the redox balance. The parameters that better evidenced COPD changes show combined variability, as illustrated in Fig. 2.

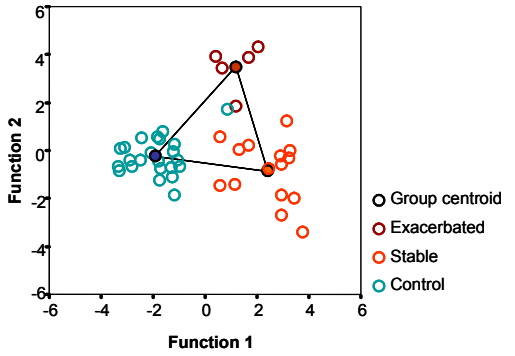

Fig. 2 – Discriminant analysis for the COPD data set established, separating the three studied groups. Retained parameters having significant correlation for sputum (S) and blood indicated in the graph.

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Assessing iron overload through skin in human metabolic disorders

T. Pinheiro, L.C. Alves, I. Guinote, R. Silva[1], R. Fleming [2], A. Barreiros [3]*

Hereditary or secondary hemochromatosis induces iron overload facilitating the occurrence of a variety of diseases, including cirrhosis, congestive heart failure, diabetes, and hepatocellular carcinoma. Skin is an accessible organ, affected by this disease and can be used to monitor body iron status. Iron metabolism indicators, such as serum ferritin concentration, total iron binding capacity and transferrin saturation, iron concentration in serum, plasma, and skin, and the determination of iron deposition in liver were assessed in nine patients with hereditary hemochromatosis (hetero or homozygoty for at least one HFE gene mutation – C282Y or H63D) along the therapeutic programme. Skin and serological evaluation was made before and after the initial phase of therapeutic (weekly phlebotomies) and six months after stopping the initial phase of the phlebotomy programme. Liver iron deposits were determined at least before and after therapy. A strong relationship between iron concentrations in skin and plasma iron and serum ferritin contents was found ($p < 0.02$). Iron deposition increase in liver, as assessed by Nuclear Magnetic Resonance, was related to ferritin concentration and transferring saturation rise in serum ($r^2 > 0.9$; $p < 0.001$) and at a less extend to increased iron concentrations in skin ($r^2 = 0.65$; $p<0.1$). Also the serum hepcidin content a peptide excreted by liver that is believed to regulate intestinal Fe absorption is being determined.

The relationship of the parameters measured for iron metabolism and organ deposition may help to a better understanding of iron pools mobilisation. In particular skin due to its accessibility may constitute an alternative marker to the disease progression and therapy efficacy. The study is supported by the projects SPDV /2004-2006 and IAEA RC 302-F1.20.19-POR-13262 2005-2007.

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Skin Permeability to Nanoparticles

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Nanoparticles of TiO_2 and ZnO are widely used in commercial sunscreens by their capacity to scatter UV wavelengths of sunlight. Skin exposure to commercial products containing nanoparticles of Ti, Zn, and Si oxides, among others and their trans-epidermal diffusion has been studied using nuclear microscopy techniques. One of the objectives of the work is to assess the percutaneous penetration depth of Ti oxides. Therefore, methodologies were adjusted to enable the validation of elemental distribution maps or profiles with high-resolution images originated in transmission mode (STIM, Scanning Transmission Ion Microscopy). The work is carried out under a European consortium EC/QLK4-CT-2002-02678.

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Bioavailability of Fe, Cu and Zn in small mammals in a pyrite mining area, South Portugal

T. Pinheiro, M.A. Barreiros [1], C. Ralheta [1], C. C. Marques [2], S. L. Gabriel [2], M. L. Mathias [2]

Mining areas are ecologically significant sources of metal contamination. Chemical analysis of soil and water can provide useful information on environmental contamination, but cannot easily assess the bioavailability and toxicity of metals in living organisms. Small mammals have been shown to be relevant in habitat contamination monitoring. The Algerian mouse, *Mus spretus,* is a widespread species across its range and has been occasionally used in some ecotoxicological studies. The biological availability of metals in mice inhabiting contaminated areas can be assessed by comparing the concentration of metals in the environment (e.g. surface water) with the corresponding body burdens of the exposed animals. This work is part of an ecotoxicological survey (project POCTI/BFE/3991/2001-2005) carried out in a mining area, included in the Iberian Pyrite belt, South Portugal. A reference area with similar environment conditions (climate and vegetation) was chosen based on the absence of known sources of contaminants. The metals present in water were determined using Total Reflection X-Ray Fluorescence and the elemental contents in liver samples were quantified using both Particle Induced X-Ray Emission and Total Reflection X-Ray Fluorescence techniques. In the mining area, the contamination of surface water with Fe, Cu and Zn is perceptible. However, only Fe accumulates in the liver of exposed animals when compared with those from the reference areas. Also, an increase in Se concentration was observed suggesting changes in the health condition of the exposed animals.

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