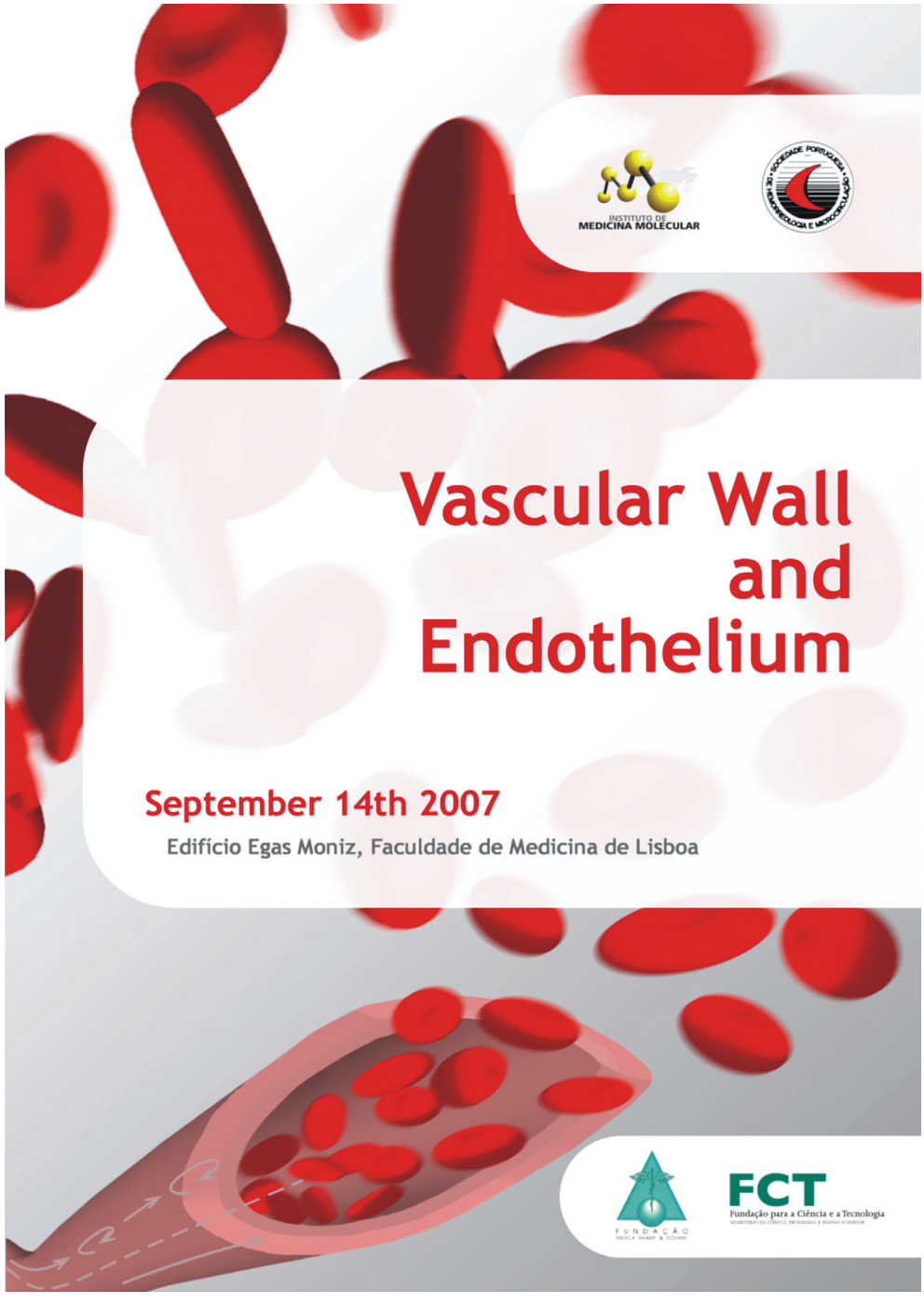
7.º Curso Avançado em Bioquímica Aplicada



PROGRAMA/PROGRAM

RESUMOS/ABSTRACTS

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PROGRAM

09h00 Participants reception

09h20 **Opening session**

09h30 - 10h30 ***1st Session***
Jean-François Stoltz (Fac. Med, Univ. Nancy)
Mecanobiology of endothelial cells
António Duarte (FMV, UTL)
Notch signalling in the regulation of arteriogenesis and angiogenesis

10h30 - 11h00 Coffee break

11h00 - 12h00 ***2nd Session***
João Barata (IMM, FML)
Leukemia cells and the bone marrow endothelium
Adélia Sequeira (IST, UTL)
Progress in computational haemorheology and haemodynamics

12h00 - 15h00 Lunch and Poster Session

15h00 - 16h50 ***3rd Session***
Sérgio Dias (IPOFG, IGC)
The importance of the vascular compartment in bone marrow function
Carlota Saldanha (FML, IMM)
Non-neuronal cholinergic mechanisms in red blood cell

16h00 *João Martins e Silva* (IMM)
Conclusions and Closing

ENDOTHELIAL CELL BIOLOGY INFLUENCE OF MECHANICAL FORCES

JF Stoltz[1,2], V. Decot[1,2], S. Muller[1], A. Kadi[1], P. Lacolley[3],P. Menu[1], D. Bensoussan[1,2]

Vascular endothelial cells form a monocellular layer on blood vessel wall with an estimated mass of 1.5 kg. The role of endothelial cells is among to control the hemodynamics through various metabolic activities affecting homeostasis, vascular tonus, blood fluidity, coagulating properties and blood cells adhesion. In other respect thousands studies have underlined the crucial role of local blood flow conditions on their properties. However, the hemodynamic forces are different according to the anatomical site and to the type of blood vessels (arteries, veins, venules).

In microcirculation, the endothelial cells in the venules are particularly active and constitute the physiological site of liquid exchange (permeability) and above all cellular transit. During critical ischemia, the post-capillary venules are deeply involved. In other respect, the properties of endothelial cells may be impaired in many diseases as atherosclerosis, hypertension, inflammation and metabolic diseases...

The endothelium is normally antithrombotic and anti-adhesive, to ensure "blood fluidity". During aggressions, the endothelium can reverse their functions by expressing stored material or by slower involvement of genes which until then were repressed.

The anti-thrombotic and "fluidifying" actions of endothelial cells are due to three types of properties:

a) vaso-regulating properties: they are controlled by the release of vasomotor components as endothelin ET-1 prostacyclin (PGI2) and nitrite oxide (NO).
b) anti-thrombotic properties: Endothelial cells express proteoglycans on their surface, including plasminogen, sulfate glycoaminaoglycans, negative-electric charges. They secrete plasminogen tissular activator (t-PA) and tissular factor inhibitor. One other fundamental propertie of the endothelium is the production and expression of thrombomodulin (a thrombin receptor). Endothelium cells also exert anticoagulant properties by other channels, such as the capture and degradation of thrombogenous substances (ADP, 5-HTP) and through the effects of active products on platelets,
c) anti-adhesive properties: in an inactive or active state the endothelial cells express adhesion molecules, that can be modulate by mechanical or biochemical stimulations.

During inflammation, the endothelial cell properties may be reversed. Variations in local shear stresses may also modify the secretion of vasomo-

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tor substances. In this case thrombomodulin, is under-regulated. In addition, the cell express tissular factor that can bind to plasma VIIa factor. Hence thrombin production may occur all the more easily as factors I and X possess binding sites on the endothelium. Regarding fibrinolysis, PAI-1, is increased and t-PA is under-regulated.

Morever, endothelial cells produce von Willbrand Factor through the Weibel Palade bodies. In the case of endothelial lesions, von Willbrand Factor will be expressed, and enhance the platelet adhesion with a binding with the platelet GPIb-IX-V complex. It was show that the expression of von Willbrand factor is regulated by the blood flow.

In other respect, the influence of local hemodynamic conditions on endothelial cells has raised increased interest over the recent years. Various studies have tried to clarify the physiology and modifications of EC during vascular diseases. Endothelial cells react to hemodynamic forces by modifying their morphology and metabolism. The morphological changes may include elongation and orientation of endothelial cells parallel to the flow direction as well as actin filament rearrangement, responsible for cellular mobility and adhesion. The metabolic changes mainly include increased prostacyclin synthesis, plasminogen tissular activator expression, differential regulation of adhesion molecule, genes expressions and ion transfer (K+,Ca++); Shear stresses also stimulate endothelial cell proliferation and migration. Various types of response of endothelial cells to the mechanical stress have been described and could be in a simplified manner classified into three types:

- early and transient increase (c-fos, c-jun, c-myc, PDGF, …),
- continuous increase in mRNA expressions (t-PA, NOS, ICAM1, ...),
- two-phase regulation: increase during the first two hours followed by a continuous decrease after the 12th hour (ET-1, PDGF-β, VCAM1, …).

This different aspects will be discussed during the presentation.

NOTCH SIGNALING IN THE REGULATION OF ARTERIOGENESIS AND ANGIOGENESIS

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The importance of the Notch signaling pathway in the regulation of vascular development and angiogenesis was initially suggested by the expression of Notch receptors and ligands in vascular endothelial cells and the observed vascular phenotypes in mutants for the Notch receptor or ligands, especially *Dll4*. In the vascular system, Notch receptors and ligands are expressed mainly on arteries, with Delta-like 4 (Dll4) being the

only ligand expressed early during the development of arterial endothelial cells and capillaries. *Dll4* null embryos die very early in development with severely reduced arterial calibre and lumen and loss of arterial cell identity. Detailed analysis of these mutants showed that the arterial defect precedes the initiation of blood flow and that the arterial *Dll4*$^{-/-}$ endothelial cells proliferate and migrate more actively. *Dll4*$^{-/-}$ mutants displayed a defective basement membrane around the forming aorta and increased endothelial cell migration from the dorsal aorta to peripheral regions, which constitute the main causes of arterial lumen reduction in these embryos. On the other hand, gain-of-function mutants, produced by cre-based recombination transgenesis, displayed grossly enlarged dorsal aortae and died before E10.5, showing a variable degree of premature arteriovenous fusion. Veins displayed ectopic expression of arterial markers. Other defects included reduced vascular sprouting, endothelial cell proliferation and migration. *mDll4* overexpression also inhibited VEGF signaling and increased fibronectin accumulation around the vessels. These phenotypic traits appear to depend on Dll4 regulation of VEGFR-2 and VEGFR1, which are respectively upregulated and downregulated in the loss-of-function mutant and *vice-versa* in the *Dll4* overexpressing embryos. Together, these results strongly suggest that Notch signalling can increase arterial stability and calibre by decreasing the response of arterial endothelial cells to local gradients of pro-angiogenic factors like VEGF.

LEUKEMIA CELLS AND THE BONE MARROW ENDOTHELIUM

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Recruitment and proliferation of endothelial cells resulting in the formation of new blood vessels from preexisting ones (angiogenesis) plays a critical role in the growth of solid tumors. Angiogenesis also occurs in hematological malignancies, such as leukemia, lymphoma and multiple myeloma. The biological and clinical implications of bone marrow endothelium interaction with hematological tumor cells are somewhat controversial. However, there is evidence suggesting the existence of putative bone marrow niches where endothelial and leukemia cells contribute with mutually beneficial stimuli that promote both an angiogenic phenotype and leukemia expansion. Factors such as SDF1 and VEGF have been implicated in the interplay between endothelium and leukemia, and may constitute targets for therapeutic intervention.

Our research focuses on the role of microenvironmental factors for leukemia cell proliferation and viability. Interleukin 7 (IL-7) is produced by bone marrow stromal and endothelial cells. While IL-7 may stimulate endothelial cells, different studies have clearly shown that it is a leukemia growth factor, particularly for T-cell

acute lymphoblastic leukemia (T-ALL). Although other cytokines from the IL-2 family, such as IL-9 or IL-15 may have similar leukemia-stimulating effects, IL-7 is clearly more potent than those, inducing striking T-ALL cell expansion in vitro. IL-7 mediates leukemia proliferation and viability by triggering the activation of PI3K/Akt(PKB) pathway leading to p27kip1 downregulation, Bcl-2 upregulation, and consequent cell cycle progression and decreased apoptosis. In turn, PI3K-dependent modulation of intracellular reactive oxygen species appears to be essential for IL-7-mediated viability of T-ALL cells.

Irrespective of the intracellular mechanisms by which IL-7 stimulates T-ALL cells, it is tempting to hypothesize the existence of bone marrow niches where stroma/endothelium-produced IL-7 promotes leukemia expansion.

PROGRESS IN COMPUTATIONAL HAEMORHEOLOGY AND HAEMODYNAMICS

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Whole blood is a concentrated suspension of formed cellular elements that includes red blood cells (erythrocytes) white blood cells (leukocytes) and platelets (thrombocytes). Blood cells are suspended in plasma, an aqueous ionic solution. In the large vessels where shear rates are high enough, it is reasonable to assume that blood has a constant viscosity and a Newtonian behaviour. However in smaller vessels and capillaries or in some diseased conditions, the presence of the cells induces low shear rate and blood exhibits remarkable non-Newtonian properties, like shear-thinning viscosity, thixotropy and viscoelasticity. We refer to[1,2] as recent review papers on mathematical models of blood rheology.

In this talk we present a short overview of some constitutive models that can mathematically characterize the rheology of blood and some numerical simulations to illustrate its phenomenological behaviour[3]. Using a mesoscopic lattice Boltzmann flow solver for non-Newtonian shear thinning fluids, we present a three-dimensional numerical study of the dynamics of leukocytes rolling and recruitment by the endothelial wall, based on in vivo experimental measurements in Wistar rat venules[4]. Preliminary numerical results obtained for a comprehensive model of blood coagulation and clot formation, that integrates physiologic, rheologic and biochemical factors will also be presented[5]. The corresponding three-dimensional simulations were obtained for a shear-thinning blood model using a finite volume semi-discretization in space and a three-stage Runge-Kutta time integration method.

Key-words: *Blood rheology, coagulation, leukocytes, shear-thinning flows, numerical simulations, lattice Boltzmann method, finite-volumes.*

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THE IMPORTANCE OF THE VASCULAR COMPARTMENT IN BM FUNCTION

Sérgio Dias
Instituto Português de Oncologia Dr. Francisco Gentil

Hematopoietic differentiation in the bone marrow requires the establishment of specific molecular and cellular interactions between hematopoietic stem cells, progenitors and committed progeny and stroma elements (endothelium, fibroblasts, adipocytes, osteoblasts), interactions which have been globally defined as representing a "niche" (or more than one). Despite significant increases in our knowledge of the composition and regulation of the "normal" hematopoietic niche, the importance of such "niches" in bone marrow diseases is still largely unknown.

Endothelial cells are one component of bone marrow niches that exert crucial functions within the bone marrow microenvironment, namely by modulating the trafficking and the terminal differentiation of hematopoietic cells. Angiogenic growth factors such as Vascular Endothelial Growth Factor (VEGF) promote the survival and modulate the hematopoietic-supporting functions of bone marrow endothelia. Conversely, abnormal production of VEGF within the bone marrow may promote endothelial proliferation/activation, and consequently affect the hematopoietic microenvironment.

In detail, we have revealed the selective inhibition of B lymphoid differentiation by VEGF, signalling via its VEGFR-1 (FLT-1) in normalcy. Recently we have also shown that in BM diseases like MDS, induction of apoptosis in the vascular lineage results in abnormal (displastic) BM. This mechanism involves an intricate cross-talk between VEGF and TNF-alpha, produced by different cells within the BM.

Taken together, the importance of the endothelial/vascular compartment in the regulation of BM functions is just now beginning to be elucidated; research in this area will certainly contribute towards the identification of novel strategies to promote adequate BM recovery/functions and also inhibit the angiogenesis processes involved in BM malignancy.

NON-NEURONAL CHOLINERGIC MECHANISMS IN RED BLOOD CELL

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There is evidence about the presence of acetylcholine (ACh) in the blood circulation coming from T lymphocytes and endothelial cells, where its synthesis occurs. "The non-neuronal cholinergic system" (NNCS) as a new paradigm emerged, and a huge scientific development exists. The NNCS has been tied with vessel wall vasomotor activity as a result of the nitric oxide production by endothelial cells induced by the blood presence of ACh. Another component of the NNCS is the enzyme, that hydrolysis ACh, the acetylcholinesterase (AChE) localized in endothelial cells, lymphocytes and erythrocyte membrane. The current knowledge on transport and consumption of oxygen in red blood cells (RBCs) was further added by the discovery that protein band 3 (erythrocyte membrane anionic channel) is involved in the exportation of nitric oxide (NO) from the RBCs stores.

The NNCS implications on the erythrocyte NO metabolism and RBC mechanical behaviour will be described and its responses explained by the signal transduction mechanism studied.

We demonstrate that red blood cells (RBCs) incubated in the presence of ACh results in the increase of the P50, S-nitrosohemoglobin, lactate values and decreased level of S-nitrosoglutathione without any changes in 2,3-biphosphoglycerate concentration.

However, the decreased glucose-6-phosphate dehydrogenase enzyme activity may explain the lactic fermentation pathway choice (NAD^+ regeneration) and the absence of RBCs ability to return the higher levels of MetHb to the Hb ferrous state when in ACh loading suspensions. Our results showed that in human erythrocytes NO translocation and mobilization occurs in presence of ACh with increased basal levels of NO, nitrites and nitrates (NOx) in erythrocytes suspensions. The presence of ACh in erythrocytes suspensions induced band 3 phosphorylated states associated with protein Giαβγ while the dephosphorylated satate interact with Giα subunity when the ACh- AChE active complex is formed. Protein band 3 plays a role in the maintenance of erythrocyte stability and shape by its interaction with cytoskeleton proteins that require protein kinase C (PKC) phosphorylation. We observed that erythrocyte deformability decreased by inhibition of PKC, especially when it was conjugated with protein tyrosine kinase, 72Syk, inhibitor plus ACh. Activation of PKC activity promote the increased of erythrocyte deformability, which it is decreased in presence of phosphotyrosine phosphatase inhibitor. Otherwise the erythrocyte deformability behaviour influenced by the degree of phosphorylation of band 3 and the presence of AChE effectors were modified by

the activity status of the PKC in a dependent way.

The RBCs responses, verified *in vitro*, to the components of the NNCS, may be considered as a RBCs homeostatic control mechanism. This hypothesis was partially observed with less deformable RBCs obtained from hypercholesterolemics patients translocate more NO when were stimulated with ACh in comparing with healthy persons.

Keywords: *Erythrocyte membrane, band 3, protein tyrosine kinases, phosphatase, acetylcholinesterase, acetylcholine, velnacrine, cytoskeleton, protein kinase C, nitric oxide, non neuronal cholinergic system.*

MECHANOTRANSDUCTION PATHWAYS IMPLICATED IN NO PRODUCTION IN ENDOTHELIAL CELLS TREATED WITH A SELECTIVE B1-ADRENERGIC RECEPTOR ANTAGONIST

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A part of vascular endothelial dysfunction are caused by an impairment in nitric oxide biodisponibility, involving resistance arteries and leading to increase the systemic blood pressure and thus to rise hypertension. Hence antihypertensive agents that reverse endothelial dysfunction and lowers blood pressure might improve the prognosis of patients with hypertension. Many studies have reported that Nebivolol® which has been shown to be a selective blocker of β1-adrenoceptors can reverse endothelial dysfunction, and so via an increase in nitric oxide release, treat hypertension disorder. In other respect, endothelial cells are known to be particularly sensitive to mechanical stimuli, as the shear stress induced by the blood flow and are able to convert it into intracellular signals such as including activation of various kinases and ionic channels, production of vasoactive substances, gene expression and phenotypical modulation. These parameters are able to induce structural modifications of the arterial wall. Mechanisms by which these mechanical forces are sensed and converted into intracellular biochemical signals are not fully understood. Mechanotransduction signal is hypothesized to occur through three major pathways involving ion channels, membrane perturbations or the cytoskeleton. Many laboratories have studied the signaling mechanism of the Nebivolol in endothelial cells. Many hypothesizes in the literature suggest that the endothelium-dependent liberation of NO induced by Nebivolol® in static conditions, is due to stimulation of β3-adrenoreceptors and oestrogen receptors and was dependent from eNOS activation so implicating PI3-K and Akt signaling pathway. Our studies have concern the endothelium-dependent liberation of NO induced by Nebivolol® under shear stress conditions. We found that Nebivolol® was more effective when associated to shear stress than in static conditions. Our current studies concern the endothelial cells mechanotransduction induced by Nebivolol® when associated with a vascular physiological mechanical stimuli.

ADVANCES IN COMPUTATIONAL HEMORHEOLOGY

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Experimental investigations conducted over the years have shown that blood can exhibit non-Newtonian characteristics like shear-thinning, viscoelasticity and thixotropy. The ability to describe the complex rheological behaviour of blood, which is determined by numerous physiological factors like plasma viscosity, rate of shear, haematocrit or level of red blood cell aggregation and deformability, among others, is of major importance in many clinical applications, where local hemodynamics plays a determinant role. A detailed discussion of different models and methods can be found in[4].

In some districts of the vascular system, like in large vessels, blood viscosity can be considered as a constant and blood flow is well described by the Navier-Stokes equations. In smaller vessels, the non-Newtonian effects are not negligible and more complex models must be used. A constitutive law, involving a nonlinear relation between the stress and the deformation gradient, is added to the system of equations.

The mathematical and numerical analysis of these complex problems can become a formidable task, both from the theoretical and the computational view points. In our presentation we show some results of blood flow simulations, using different numerical techniques, to study hemodynamical and hemorheological circulation effects[1,2,3,5].

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LOCALIZED HYDRODYNAMICS OF CLUSTERING LEUCOCYTES

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The recruitment of leukocytes to the endothelial walls is intensively investigated both experimentally and through three dimensional computer simulations.

The shear dependent viscosity has been obtained from measured values in post-capillary venules of Whistar rats cremaster muscle. Localized velocity fields and shear stresses on the surface of leukocytes and near vessel wall attachment points have been computed and discussed for a cluster of recruited leukocytes under generalized Newtonian blood flow with shear-thinning viscosity.

We have observed one region of maximum shear stress and two regions of minimum shear stress on the surface of the leukocytes close to the endothelial wall. This suggests that the accumulation of selectins attains a minimum value in two regions, rather than in one region, on the surface of the leukocytes.

We have also verified that the collective hydrodynamic behavior of the cluster of recruited leukocytes establishes a strong motive for additional leukocyte recruitment. The study suggests that the influence of the leukocytes rolling on the increase of the endothelial wall shear stress may support the activation of more signalling mediators during inflammation[1,2,3].

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COUPLING MULTISCALE FLUID-STRUCTURE INTERACTION MODELS FOR BLOOD FLOW SIMULATIONS

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Over the last years, mathematical modelling and numerical simulations of blood flow have gained a great relevance in the understanding of the human cardiovascular system, in particular the origin and development of cardiovascular diseases[1]. However, modelling the human circulatory system remains a very difficult and challenging task because of its complexity and heterogeneity, both geometrically and functionally. In particular, realistic numerical simulations of blood flow in arteries can not be performed without taking into consideration the link between local and global phenomena[1,2]. Moreover, blood flow is characterized by pulse waves due to the fluid-structure interaction (FSI) between blood and the vessel wall, which should be properly captured by the numerical model[2,3].

The geometrical multiscale modelling of the cardiovascular system was introduced to deal with this complexity and diversity. It consists of a hierarchical description, in which the different parts of the circulatory tree are approximated at different dimensional scales, 3D, 1D and 0D, corresponding to different levels of desired accuracy[2]. At the higher level are the three-dimensional (3D) models. They describe very accurately the blood flow velocity and pressure fields, but in practice they are applied only to relatively small computational domains. This fact is linked to their computational cost, the impossibility (at least for now) of representing the whole 3D geometry of the circulatory system, and the fact that detailed information is usually needed only in specific regions of interest, such as bifurcations or stenosed vessels. On the artificial sections generated by the 3D domain truncation, one can account for the remaining parts of the cardiovascular system by using measured data or by means of simpler, reduced one-dimensional (1D) or lumped parameter (also called 0D) models. They are usually obtained by making simplifying assumptions and performing averaging procedures on the 3D model. In particular, the 1D models are described by hyperbolic systems of PDEs and, despite having a lower level of accuracy compared to the full 3D model, are still able to capture effectively the pulse waves characteristic of blood flow. Coupled to the 3D detailed problem, the 1D models can act as absorbing (or far field) boundary conditions. Moreover, due to their low computational cost, they can be used to simulate large parts of the arterial tree.

One of the challenging tasks in using the geometrical multiscale approach is the setting of proper coupling conditions to exchange quantities such as the flow rate or the mean pressure at the interfaces between different models. When using compliant geometries for the 3D simulation there is the additional request of having an appro-

priate condition for the arterial wall deformation at the interface[2,3].

In this study we focus on the coupling between the three different types of models, with particular emphasis on the coupling between 3D and 1D models[2,3]. The 3D model consists of the Navier-Stokes equations for incompressible and Newtonian fluids, since we apply it in medium to large vessels where blood is assumed to be Newtonian, coupled with a model for the vessel wall. We discuss different strategies to impose the continuity of the flow rate, mean pressure and area at the interface between the 3D and 1D models. Furthermore, we consider an anatomically 3D realistic compliant model of a human carotid bifurcation coupled with reduced models at the downstream sections, including a 1D network representation of the circle of Willis[2,4].

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HEMORHEOLOGIC PARAMETERS IN ANIMAL MODELS OF HYPER AND HYPOCHOLESTEROLEMIA AND HYPERTENSION

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Animals are often use nowadays as scientific investigation models for many kinds of diseases and the experimental results obtained have served as the basis for many clinical trials. Different experimental are animal models (EAM) are described in the literature and choosing an appropriate model to answer our questions is always a challenging. In order to contribute to a better understanding of the biophysiological properties underlying the EAM we performed a hemorheological characterization of different EAM namely, hyper/hypocholesterolemia and systemic arterial hypertension.

The hypocholesterolemia model was achieved with a daily ingestion of low fat milk enriched with phytosterol esters, and a hypercholesterolemia profile was obtained with the ingestion of low fat milk with no food ingredient addition. We demonstrated that the used of phytosterol as food ingredient reduces the plasma concentrations of cholesterol and LDL-cholesterol, not affecting the HDL-cholesterol levels. During the experiment no clinical changes and no significant differences in growth, food or milk comsumption were observed. Blood and plasma viscosity, erythrocyte de-

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formability and membrane fluidity were determined in all animal groups. The hypercholesterolemic profile is characterized by a decrease in the blood viscosity, in the membrane fluidity and in the erythrocyte deformability and no changes in the plasma viscosity. In the hypocholesterolemic profile achieved with the ingestion of phytosterols esters as food ingredient no significant changes were observed in the hemorheological parameters studied.

The animal model of systemic arterial hypertension is achieved with a daily ingestion of a NO sintase (NOS) inhibitor (L-NAME) for 21 consecutive days which results in an increase of the systolic and diastolic blood pressures. Concerning the hemorheological parameters determined we observed that NOS inhibition has no effect on the erythrocyte membrane fluidity, but increases the erythrocyte deformability. The higher erythrocyte ability to deform may be a compensatory mechanism of the NO decreased levels, known as a vasodilator. Due to the blood and plasma viscosities, also determined, no variations were observed in the blood viscosity, but a higher plasma viscosity is obtained in this hypertensive model.

The hemorheological characterization of an animal model is important both for its use in further experiments and its possible basis for clinical trials.

ENDOTHELIAL FUNCTION IN NEWBORN INFANTS FROM PREECLAMPTIC PREGNANCIES

Cristina Catarino[1,2], Irene Rebelo[1,2], Luís Belo[1,2], Susana Rocha[1,2], Elisabeth Bayer Castro[1,2], Belmiro Patrício[3], Alexandre Quintanilha[2,4], Alice Santos-Silva[1,2]

Preeclampsia (PE) is a characteristic hypertensive disorder of human pregnancy that is potentially dangerous for both mother and fetus. It is widely accepted that the fibrinolytic system is altered in PE, and is likely to result from the underlying endothelial dysfunction observed in this syndrome. A significant increase in PAI-1 antigen as well as in tPA antigen has been observed in PE and these may work as markers of endothelial dysfunction.

Our aim was to evaluate some hemostatic variables in normal and PEc pregnancies at delivery, both in maternal and umbilical cord blood (UCB). We measured the antigen plasma levels of tissue plasminogen activator (tPA) and of plasminogen activator inhibitor type 1 (PAI-1), both markers of hemostatic and endothelial function disturbances, and fibrin fragment D-dimer.

Maternal blood from uncomplicated (n=42) and PEc pregnancies (n=44) were collected before delivery, and UCB immediately after delivery of the placenta. We found significantly higher values for PAI-1 and tPA in PEc women when com-

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pared with normal pregnant women, but no significant difference was found for D-dimer. In UCB, only tPA was significantly higher in PEc cases. In women with PE, proteinuria (marker of PE severity) correlated positively and significantly with tPA (r=0.44, P= 0.003) and PAI-1 antigen levels (r=0.58, P< 0.001). An inverse relationship between maternal tPA antigen levels and fetal birth weigh in PE (r=-0.63, P< 0.001) was also observed.

In summary, tPA and PAI-1 levels are higher in PEc women, suggesting endothelial dysfunction, and correlate with the severity of PE. Furthermore, these PEc hemostatic changes seem to have impact in fetal circulation. We suggest that tPA may be a good marker of fibrinolytic impairment and of endothelial dysfunction, particularly in the maternal circulation, and that the impact of raised tPA levels in the neonates from PEc mother deserves further studies.

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ALTERED ERYTHROCYTE MEMBRANE BAND 3 PROFILE IN CHRONIC RENAL FAILURE PATIENTS UNDER HAEMODIALYSIS

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Red blood cells (RBC) present a limited biosynthesis capacity, accumulating physical and chemical changes, along its life span. Proteolytic cleavage, clustering or exposure of unusual epitopes of band 3, a major integral protein of RBC membrane, triggers the binding of specific anti-band 3 autoantibodies and complement activation, marking RBC for death. An abnormal band 3 profile [% of band 3 monomer; high molecular weight aggregates (HMWAg); proteolytic fragments (Pfrag)] has been associated with RBC damage/aging in inflammatory conditions associated with oxidative stress. Chronic renal failure (CRF) has also been associated with both inflammation and oxidative stress. A

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deficient renal erythropoietin secretion underlies the development of an anaemia, usually corrected by therapy with recombinant human erythropoietin (rhEPO). However, about 25% of the patients do not respond to this therapy.

Our aim was to study changes in RBC membrane band 3 profile, as a cumulative marker of RBC changes, in chronic renal failure (CRF) patients under haemodialysis and recombinant human erythropoietin (rhEPO) therapy and its linkage with resistance to this therapy.

We studied 63 CRF patients, 32 responders and 31 non-responders to rhEPO therapy, and 26 healthy individuals matched for age and gender. We evaluated the band 3 profile and membrane-bound haemoglobin (MBH). Total serum bilirrubin, glutathione peroxidase and superoxide dismutase activities, RBC count, haematocrit, haemoglobin concentration, haematimetric indices and reticulocyte were also evaluated. CRF patients presented anaemia, slightly regenerative, as showed by the decreased RBC count, Hb and haematocrit, alongside with an increased reticulocyte count, RPI and RDW values. A rise in GPx and a trend to lower values of MBH were also found in CRF patients. A positive correlation was found between Pfrag and, Hb and haematocrit. When comparing the haematological data between the two groups of CRF patients, we found that non-responders patients were more anaemic, and presented a statistically significant decrease in Pfrag, and a trend for a rise in MBH, suggesting a higher RBC damage.

Our data suggest that band 3 profile seems to be a good marker of erythrocyte changes in CRF patients. These changes seem to be associated with a younger RBC population, but also with a rise in RBC damage, which is enhanced in non-responders CRF patients. Band 3 profile could be used as a marker of RBC changes in these patients and in the understanding of the mechanism of resistance to rhEPO therapy.

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FIBRINOLYTIC ACTIVITY AND VASCULAR ACCESS IN CHRONIC RENAL FAILURE PATIENTS UNDER HAEMODIALYSIS

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Cardiovascular disease events are the cause of death in chronic renal failure (CRF) patients. Disturbances in coagulation and fibrinolysis have been reported in patients with chronic uremia, and are known to contribute to the pathogenesis of cardiovascular diseases. However, studies about coagulation and fibrinolysis in CRF patients under regular hemodialysis have yielded conflicting results. Some studies reported a suppressed fibrinolysis and others an increased fibrinolysis. These controversial results may be related to the type of vascular access – central venous dialysis catheter or AV-fistula – chosen for the hemodialysis procedure, and may associate a different risk of thrombosis.

Our aim was to study the relationship between fibrinolytic activity and the type of vascular access in haemodialysis patients. We measured the circulating antigen levels of plasminogen activator inhibitor type-1 (PAI-1) and tissue plasminogen activator (tPA); D-dimers were also evaluated. This study was performed in 50 CRF patients under regular haemodialysis, 11 with central venous dialysis catheter and 39 with AV-fistula, and in 25 healthy controls.

Compared with controls, CRF patients presented significantly lower levels of tPA and with higher levels of D-dimers. In CRF patients, the levels of D-dimers correlated positively and significantly ($r=0.359$, $p=0.01$) with rhEPO doses (rhEPO/Kg/week) and negatively with haemoglobin levels ($r=-0.335$, $p=0.017$). When comparing the two groups of CRF patients, we found that those with central venous catheter vascular access presented a statistical significant rise in D-dimer and tPA plasma levels. No difference was found between the two groups of patients concerning the plasma levels PAI-1.

Our results showed an altered haemostasis in CRF patients, as suggested by the rise in D-dimer, an index of fibrin turnover and intravascular thrombogenesis. The increased levels of D-dimer and tPA in CRF patients, particularly in those using central venous dialysis catheters, led us to propose a relationship between the type of vascular

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access chosen for the haemodialysis procedure, and the risk of thrombogenesis. It seems reasonable to assume that these patients present an increased risk for cardiovascular disease events.

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EFFECTS OF ACETYLCHOLINE ON NO TRANSLOCATION IN ABNORMAL AND MANIPULATED RED BLOOD CELLS

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Nitric oxide (NO) is known to be an important vasodilator messenger interfering in a number of physiological and pathophysiological processes. We have verified that in some diseases red blood cells have hemorheological dysfunctions, such as, changes on erythrocyte deformability and erythrocyte aggregation. We also demonstrated that the presence of acetylcholine (ACh, natural substrate of acetylcholinesterase enzyme) induce changes on the healthy erythrocytes vasomodulation role.

The aim of this study was to access the ex vivo response of NO translocation in abnormal red blood cells after ACh 10 µM stimulation and to know if this action could be related to intraglobular nitrosylated molecules and phosphorylated/dephosphorylated protein band 3 by, tyrosine kinase and phosphatase proteins, respectively.

For this propose we determined NO concentration, by an amperometric method on in vitro erythrocytes suspensions from patients with different types of diseases (drepanocytosis (n=9), renal transplantation (n=36), chronic venous peripheral disease (n=22), arterial hypertension (n=20) hypercholesterolemia (n=102) coronary ischemia (n=34) and delirium associated with stroke (n=115) comparing with NO levels achieved on erythrocytes of healthy persons (n=27). We stimulate erythrocytes of healthy persons with tyrosine kinase and tyrosine phosphatase proteins inhibitors ($p72^{syk}$ inhibitor 10 µM and calpeptin 10 µM, respectively) for 30 minutes at 37 ºC and also measured NO levels.

NO concentration values obtained were: 2,0 ± 0,8 nM (control), 4,5 ± 1,4 nM (drepanocytosis, P<0,001), 2,2 ± 0,9 nM (renal transplantation), 2,3 ± 0,6 nM (chronic venous peripheral disease), 2,8 ± 1,1 nM (hypertension, P=0,005), 2,3 ± 0,7 nM (hypercholesterolemia), 2,8 ± 0,9 nM (coronary ischemia, P=0,001) and 2,6 ± 0,8 nM (delirium associated with stroke,

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P=0,001). We observed the most significant change on NO translocation with drepanocytosis erythrocytes samples which could be a positive factor for the compromised tissue oxygenation in this kind of anaemia. On all different pathologies studied there were a tendency to increase NO erythrocyte translocation. On the other and, manipulated red blood cells shows that in the presence of ACh, forming an acetylcholinesterase active enzyme complex, increase NO levels. The addition of $p72^{syk}$ inhibitor, protein band 3 at partially phosphorylated state, reveal a decrease of NO concentrations with ACh. In presence of calpeptin and ACh, band 3 being totally phosphorylated, we observed an increase of NO levels.

In conclusion, human erythrocytes of different diseases have diverse physiological responses to ACh stimulation that leads to changes on NO mobilization mechanisms. When we manipulated the phosphorylated/dephosphorylated states of band 3 protein, the results reveal that there are changes on erythrocytes NO translocation in the presence of acetylcholinesterase substrate. The results demonstrated the key-role of acetylcholinesterase effector-associated band 3 phosphorylation/dephosphorylation pathway in the mobilization of the erythrocyte NO stores, which might facilitate to understand some intracellular erythrocyte-dependent events underlying hypoxic conditions on microcirculation disease. This suggests a future target for vasodilator therapeutic action on a microcirculatory network, by controlling the tissue oxygenation, that could be damaged by different sorts of stimulus.

IDENTIFICATION OF THE LINKAGE BETWEEN G PROTEINS AND ERYTHROCYTE PROTEIN BAND 3

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Circulating acetylcholine (natural substrate of acetylcholinesterase) is able to modulate the microcirculatory blood flow by controlling nitric oxide (NO) intracellular mobilization, its metabolism (NO_x) and its release from erythrocytes. In reverse, velnacrine maleate plays a competitive role as an acetylcholinesterase (AChE) inhibitor decreasing NO-mediated erythrocyte responses. A possible hypothesis

In our previous studies we hypothesis that a possible response's mechanism lies on the NO translocation among nitrosylated molecules through a protein G linked to band 3 protein. Band 3 phosphorylated/dephosphorylated states are processed by major tyrosine-kinases (PTK) and phosphotyrosine-phosphatases (PTP).

So we intend to identify the G protein form that could be linked to the protein band 3 and to know each protein G sub-units (α,β,γ) are related to the activation or inhibition of acetylcholinesterase and phosphorylation

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band 3 states. For this propose we made Western blotting analysis using primary antibodies to different protein G sub-units such as anti-protein G_{β}, anti-protein $G\alpha i_{1/2}$, anti-protein Gαi3, anti-protein $G\alpha_{i3}/G\alpha_{0}$, anti protein $G\alpha_{S}$ and anti-protein $G\alpha_{q/11}$. We could identify on erythrocytes membrane solubilised extracts possible linkage between protein $G\alpha_{i1/2}$ and/or protein G_{β} with protein band 3. The results were then confirmed by immunoprecipitation of this two protein G sub-unit with following analysis by Western blot using antibodies against protein band 3 (C-terminal) and band 3 (N-terminal).

From all the samples studied we could concluded that G protein sub-units $G\alpha_{i1/2}$ and G_{β} could be linked with band 3 C-terminal site and only $G\alpha_{i1/2}$ are bonded with band 3 N-terminal. The connection between sub-unit G_{β} and band 3 at C-terminal was not seemed. Moreover when erythrocyte acetylcholinesterase was stimulated with acetylcholine and when is present with PTK inhibitors there was an increase of the expression of the linkage between $G\alpha_{i1/2}$ – Band 3 (C- and N-terminal) and G_{β} – Band 3 (C -terminal). These two conformational states of G protein sub-units seem to be related with the phosphorylation band 3 protein states.

Heterotrimeric G proteins transduce signal transduction pathways; moreover it is known that stimulation of Gi results in inhibition of adenylyl cyclase and ATP release from these cells. So human erythrocyte $NO_{(x)}$ mobilization levels could occur under the influence of AChE effectors by mechanisms related to the degree of band 3-phosphorylation and activation of adenylyl cyclase. These events underlying NO translocation/mobilization changes may occur on microcirculation disease, a target upon which novel coadjuvant drugs may become accessible.

OXIDATIVE STRESS, ENDOTHELIAL DYSFUNCTION AND VASCULAR DISEASE ON OBESITY AND PENILE ERECTILE DYSFUNCTION

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Different cardiovascular risks factors are associated with fewer endothelial nitric oxide bioavailability and endothelial dysfunction. Obesity is one of the Occidental diseases that have an improved of cardiovascular morbility and mortality. Obesity and penile erectile dysfunction are causes of an oxidative stress induced by oxygen reactive species and free radicals production that leads to endothelial dysfunction and vascular disease. Nitric oxide is an important vasodilator messenger interfering in these diseases. So we proposed to study possible changes on biochemical and hemorheological parameters on Obesity Women (n=24) and penile

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erectile dysfunction (n=18) comparing with the levels achieved of healthy persons (control, n=10). We quantified nitric oxide levels by an amperometric method, acetylcholinesterase activity, blood viscosity, plasma viscosity, erythrocyte aggregation (5 and 10 s), erythrocyte deformability and fibrinogen concentration. The obtained NO concentration values were: 1.66 ± 0.44 nM (control), 2.1 ± 0.43 nM (obesity, P = 0.027) and 1.90 ± 0.43 nM (erectile dysfunction). Fibrinogen concentration significant increase in both pathologies studied (292.7 ± 54.3 mg/dL for obesity (P = 0.0004) and 289.6 ± 93.0 mg/dL for erectile dysfunction (P = 0.033) vs. 206.7 ± 23.8 mg/dL for control group). Erythrocyte aggregation levels at 10 s are significantly augmented in both pathologies (P = 0.012 for obesity and P = 0.05) for erectile dysfunction) but only obesity erythrocyte aggregation levels significant increase at 5 s (P = 0,002). Also, blood viscosity of erectile dysfunction group significant increase the ones of control group (3.9 ± 0.4 mPa.s vs 3.3 ± 0.4 mPa.s (control), P = 0.003). All other biochemical and hemorheological parameters do not showed statistically significant variation.

In conclusion, both diseases produce biochemical and hemorheological disorders that could be markers of future therapeutic action on vascular and microcirculatory dysfunction, by controlling tissue oxygenation.

HAEMORHEOLOGICAL CHANGES DURING RECOMBINANT HUMAN ERYTHROPOIETIN THERAPY IN A RAT MODEL OF RENAL FAILURE PRODUCED BY PARTIAL NEPHRECTOMY

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Erythropoietin (EPO), a haematopoietic growth factor produced by kidneys, stimulates the proliferation, differentiation and maturation of erythroid cells. Chronic renal failure (CRF) patients develop anaemia due, mainly, to the low production of EPO by kidneys. To treat this anaemia, recombinant human EPO (rhEPO) therapy is currently used in these patients.

The aim of this work was to study the effect of rhEPO therapy on hae-

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morheological parameters, by using a rat model of CRF induced by partial (¾) nephrectomy.

The study used adult male Wistar rats and was performed in three groups: a control one (n=6) and in two groups with induced chronic renal failure (n=9), being one of them submitted to rhEPO therapy (n=4). CRF was induced by a two-stage (¾) nephrectomy: first, about half of the left kidney was removed by left flank incision and, one week later, the right kidney was removed through a right lateral flank incision. After a three weeks stabilization period, four animals start rhEPO therapy (Recormon, Roche Pharmaceuticals, Auckland, New Zealand) in a dose of 50 IU/Kg/week, during 12 weeks. Blood samples from the control group were collected at the beginning and at the end of the experimental procedure and from CRF rats at 3, 5, 8, 12 and 15 weeks after surgical partial nephrectomy. Haemorheology and renal function were evaluated.

Three weeks after the ¾ nephrectomy, a statistically significant increase in serum urea (71.00 ± 2.66 vs 41.00 ± 0.68 mg/dL, $p<0.05$) and creatinine (0.828 ± 0.036 vs 0.412 ± 0.019 mg/dL, $p<0.05$) concentrations were found. This increase in renal function markers remained high along the 12 weeks of experimental procedure.

Comparing with controls, rhEPO treated rat have showed a statistically significant progressive increase in haemoglobin (Hb), haematocrit (Ht), red blood cells (RBC) count, mean cell volume (MCV), mean cell Hb (MCH) and red cell distribution width (RDW), showing at 12 weeks an inverse change, though still presenting significant higher values; a decrease in platelet counts, during the first 9 weeks of rhEPO therapy. When comparing haemorheological data from non-treated CRF and controls, we found only a trend to increased MCV and MCH values and a decrease in reticulocyte count. Comparing the two groups of CRF rats, we found that rhEPO treated rats presented significantly higher values in RBC, Hb, Ht and RDW. In both groups of CRF rats, at five weeks, there was a decrease in their values, showing at the end a significantly lower value when compared to controls. No consistent alterations were found in white blood cells in CRF rats, with or without rhEPO therapy.

In conclusion, partial nephrectomy seems to be a suitable methodology to induce CRF in rats and to study erythropoiesis biology. The rhEPO therapy is associated with an increased erythropoietic stimulation (increase Hb, Ht, RBC count and RDW) and a decrease in platelet count.

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KDR – A NUCLEAR SIGNALING PROTEIN

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The vascular endothelial growth factor receptor-2 (VEGFR-2, KDR) is the major mediator of several physiological and pathological effects of VEGF on endothelial cells (ECs). As a membrane receptor, KDR activates many proteins in response to VEGF that will propagate a signal leading to several cellular functions such as migration, proliferation, survival and permeability. However, we recently showed that KDR is not only a membrane protein but presents a clear expression in the nucleus of endothelial cells, which is essential for EC recovery following wounding.

So, the overall objective of this study is to investigate the role of KDR as a nuclear protein. For this purpose, HUVEC overexpressing KDR were established and our results suggest that KDR may modulate the expression and activity of several transcription factors.

Furthermore, we observed that the phosphorylation of KDR in tyrosine residues is crucial for its nuclear internalization and according to these findings we constructed several tyrosine to phenylalanine point mutants fused or not to GFP in order to examine which tyrosine residues on KDR are involved in KDR internalization.

VASCULATURE ACTIVATION AND TUMOR RE-GROWTH AFTER RADIOTHERAPY

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Since tumor growth is angiogenesis-dependent, detailed molecular and cellular studies are thus needed to understand the parameters implicated in the interactions between the tumor and vasculature compartment with the objective of improving therapeutic strategies, not only for cancer treatment but also for preventing recurrence.

In this context, the possible pro-angiogenic effects of radiotherapy on tumor vasculature, activating or promoting resistance on the endothelial cells (ECs), has been poorly characterized.

In the present study, while investigating the pro-angiogenic effects of low doses of irradiation in the vasculature, our results suggest that low doses of irradiation below 1.0 Gy are able to induce a pro-angiogenic response to wound healing without affecting cell survival or proliferation.

In order to confirm a differential response of ECs to low doses of radiation at molecular level, lung human microvascular ECs (HMVEC-L) were irradiated at 0.5 Gy and the level of tyrosine phosphorylation was

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analysed. Our results show that low doses of ionizing radiation are responsible for an increase of tyrosine phosphorylation level and several signalling proteins are activated. In the future, we propose to identify the mechanisms whereby low doses of irradiation induce a pro-angiogenic response in the vasculature in order to know in which way this process may be involved in tumor re-growth.

THE MODULATION OF CYCLIC NUCLEOTIDE LEVELS AND PKC ACTIVITY BY ACETYLCHOLINESTERASE EFFECTORS IN HUMAN ERYTHROCYTES

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Background: The non-neuronal cholinergic system, modulated by acetylcholine, is widely distributed in biological systems, contributing to organ and blood homeostasis. In human erythrocytes, acetylcholine has been proved to modulate several crucial phenomena as of rheology parameters and nitric oxide mobilization, with involvement of membrane-bound acetylcholinesterase. The influence of this dual system on signalling transduction pathways involving cAMP/cGMP and protein kinase C (PKC), still unknown, was studied here.

Methods: From blood samples of 15 healthy donors, erythrocyte suspensions (ES) were prepared and incubated with acetylcholinesterase substrate (acetylcholine) and inhibitor (velnacrine maleate), and with adenylate cyclase / guanylate cyclase inhibitors. The levels of cAMP/cGMP and PKC activity were determined afterwards by using enzyme immunoassay kits and a spectrofluorimetric method employing a fim-1 diacetate specific fluorescent probe, respectively.

Results: The presence of the acetylcholine in ES increases cGMP and decreases cAMP, at variance, velnacrine enhances both messengers. Inhibited guanylate cyclase led to lower cGMP but higher cAMP with both effectors. By turn, inhibited adenylate cyclase let to lower cAMP with both effectors and decreased cGMP with velnacrine. Regarding to the PKC activity we observed a significant decrease when guanylate cyclase inhibitor is present and no significant changes occur in presence of adenylate cyclase inhibitor. The simultaneously erythrocyte incubation with adenylate cyclase inhibitor and acetylcholinesterase effectors induced an increase of PKC activity. The same was verified when guanylate cyclase inhibitor was present instead of adenylate cyclase inhibitor.

Conclusion: Acetylcholinesterase is involved in the signalling cascades involving second messengers, and a cross-talk mechanism con-

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cerning PDE III blockade by cGMP might be on its basis. Increase of PKC activity and some conformational changes seems to occur when adenylate cyclase is inhibited and were diminished when guanylate cyclase enzyme was repressed. The influence of acetylcholinesterase effectors on erythrocyte PKC activity seems to be relevant and could be a good peripheral biochemical marker of some neurological disturbances such as on Alzheimer's disease.

UPTAKE OF LDL-CHOLESTEROL IN HUMAN ENDOTHELIAL CELLS UNDER SHEAR STRESS: A FORSTER-TYPE RESONANCE ENERGY TRANSFER (FRET) AND FLUORESCENCE CORRELATION SPECTROSCOPY (FCS) STUDY

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Numerous studies on the pathogenesis of atherosclerosis suggest that several steps are involved, such as lipid accumulation in the artery wall resulting from transendothelial uptake of LDLCholesterol, followed by LDL oxidation and uptake by monocytes/macrophages and smooth muscle proliferation. In this work, we studied the effect of shear stress on the kinetics of internalization of native LDL in human endothelial cell.

Methods: FRET and FCS techniques were performed by using Confocal microscopy. Native LDL was labeled with two carbocyanine dyes, 1,1'-dioctadecyl-3,3,3',3'- tetramethylindocarbocyanine perchlorate (DiO) as donor and 3,3'-dioctadecyloxacarbocyanine perchlorate (DiI) as receptor. FCS techniques allow us to evaluate the yield of the double labeling of the LDL-cholesterol with DiI and DiO, and FRET measurements enable us to follow the degradation of the LDL in the cells. Confluent human umbilical vein cells (HUVEC) monolayer grown on chamber slides were incubated with a culture medium (containing DiI-DiO-LDL, 10µg/mL final concentration) either in static conditions or subjected to a laminar flow (1 Pa) at 37°C under a Confocal Laser Scanning Microscope (SP2 – AOBS Leica, Germany) for 0 to 4 hours.

Results/discussion: Results show that: (1) the yield of the LDL labeling was about 30%; (2) the possibility to evaluate the kinetics of LDL endocytosis in living single cells; (3) the degradation of LDL was depending of the shear conditions and shear stress induced changes in the kinetic of uptake and degradation.

In accordance with increasing knowledge and understanding, and regarding all available data, we conclude that confocal microscopic study, FRET and FCS could be useful methods to establish the basic and clinical studies of LDL uptake in atherosclerosis. However, further stud-

ies need to be performed to elucidate the molecular mechanism by which shear stress modulates the expression of LDL receptors.

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BICUSPID AORTIC VALVE AND AORTIC ARTERIAL WALL

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Introduction: Bicuspid aortic valve occurs in 1-2% of general population. The association of bicuspid aortic valve with aortic valve disease (stenosis, regurgitation) and aortic wall pathologies (coarctation of aorta, aortic aneurism, aortic dissection) is well known. Ethiopathogenic processes leading to those associations are yet under research.

Methods: A revision of the literature is made and potential ethiopathogenic factors for the supracited associations are refered.

Results: The following possible causative aspects were found in the literature:

– genetic factors
– developmental anomalies
– structural and biomolecular anomalies of the aortic wall (extracelular matrix, apoptoic processes, medial characteristics).

Conclusion: Given the fact that bicuspid aortic valve is a frequent condition in general population and that 1/3 of those "patients" will need farther medical/surgical treatment associated to its cardiovascular morbidity, these is undoubtly an open field for further investigation.

ANGIOGENESIS IN BREAST CARCINOMAS WITH DIFFERENT EXPRESSION PROFILES

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Angiogenesis is a complex multistep process required for tumour growth and metastasis. It involves endothelial cell migration and proliferation, microvessel differentiation and anastomosis, and extracellular matrix remodelling.

Studies have shown that endoglin (CD105) is involved in the development of blood vessels and that it re-

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presents a specific marker of neovascularization of several types of tumours. Endoglin is a cell-surface glycoprotein recently identified as an optimal indicator of the proliferation of human endothelial cells. Furthermore, its expression has been correlated with poor prognosis in breast cancer patients. Despite these evidences, the relationship between angiogenesis, assessed by the immunohistochemical expression of endoglin, and the molecular subtypes of breast carcinomas is not clear.

Microarray profiling has classified invasive breast tumours into different subtypes, based on the expression of two molecular markers: ER and HER2. Thus, tumours can be classified as: Luminal A type (ER+/HER2-), Luminal B (ER+/HER2+), basal-like (ER-/HER2-) or HER2-overexpressing carcinomas (ER-/HER2+).

We have studied a cohort of 161 cases of invasive breast carcinomas, collected from the archives of the Pathology Department of the Federal University of Santa Catarina, Florianópilis, Brazil. The samples were immunostained against endoglin in whole tissue and had been previously tested for ER and HER2 status in Tissue Microarrays.

We have found that the expression of endoglin is higher in the basal-like tumours (the mean value for stained vessels is 28,8), in comparison with the other molecular subtypes (27,3 in HER2-overexpressing carcinomas, 24,9 and 18,6 for luminal A and luminal B, respectively). However, these differences are not statistically significant (p=0,23).

Here, we have shown that endoglin displays a universal expression and that there are not significant differences in the angiogenic index among the molecular subtypes of breast cancer. Therefore, the use of an anti-angiogenic therapeutic approach can be valid in all subtypes of breast cancer.

MODULATION OF ERYTHROCYTE DEFORMABILITY BY PKC ACTIVITY

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Protein Band 3 is the protein of erythrocyte membrane in highest quantity. It is phosphorylated/desphosphorylated by phosphotyrosine kinases (PTK)/phosphotyrosine phosphatase (PTP), acetylcholine (ACh) and velnacrine are protein band 3 effectors by intermediate of Acetylcholinesterase (AChE). Besides its role in erythrocyte metabolism, protein band 3 plays a role in the maintenance of erythrocyte stability and shape by is interaction with cytoskeleton proteins that require protein kinase C (PKC) phosphorylation.

At this point our question is: how does PKC activity modulate erythrocyte deformability in absence and presence of erythrocyte protein band 3 effectors of it phosphorylated degree?

To answer this we used several inhibitors like AMGT, Syk, Calpeptin and Chelerythrine Chloride, respectively inhibitors of PTK (Lyn and p72 syk), PTP and PKC, in presence and absence of band 3 effectors. We also measured in whole blood suspensions glucose, 2,3-BPG, osmolality, pH, ionogram, tHb, O2Hb and p50, and erythrocyte deformability and aggregation.

We observed that erythrocyte deformability was affected by inhibition of PKC, causing a decrease in this, especially when it was conjugated with Syk plus ACh. We propose that PKC activity is essential for maintenance of erythrocyte deformability and the degree of phosphorylation of band 3 plays a role to this effect.

These results may contribute to establish a relation between cytoskeleton proteins, which require activation by PKC activity, and band 3 phosphorylation, to better understand some diseases caused by deficiency in proteins that could be involved in this mechanism like band 3, 4.1, 4.2, spectrin-actin and several others.