RESUMOS DA REUNIÃO CONJUNTA

Sociedade Portuguesa de Hemorreologia e Microcirculação

Núcleo de Biologia Vascular da Sociedade Portuguesa de Angiologia e Cirurgia Vascular

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HEMORREOLOGIA, HEMOSTASE E INFLAMAÇÃO NA PATOLOGIA VASCULAR- Da investigação à prática clínica

1. ADIPONECTIN AS AN INDEPENDENT PREDICTOR OF TISSUE PLASMINOGEN ACTIVATOR LEVELS IN PATIENTS UNDER HEMODIALYSIS

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Introduction: Recent investigation has given particular attention to the study of tissue type plasminogen activator (t-PA) in hemodialysis (HD) patients. It has been reported that the use of recombinant t-PA instead of heparin once weekly, as compared with the use of heparin three times a week, significantly prevented dialysis catheter malfunction. Furthermore, the thrombolytic treatment of acute stroke in hemodialysis patients frequently involves the use of recombinant t-PA. Adiponectin has been noted as an important antiatherogenic, antidiabetic and anti-inflammatory protein.

Aim: To evaluate the association of tissue type plasminogen activator (t-PA) levels with clinical data of patients under hemodialysis (HD) and with several variables potentially related with endothelial (dys)function.

Methods: In a cross-sectional study involving 189 Portuguese HD patients we measured circulating levels of t-PA, lipids, oxidized-LDL (Ox-LDL), interleukin (IL)-6, C-reactive protein (CRP), adiponectin, plasminogen activator inhibitor type 1 (PAI-1) and fibrin fragment D-dimers.

Results: In all patients, t-PA correlated inversely and significantly with adiponectin, and HDL-cholesterol, and positively and significantly with age, body mass index, PAI-1, IL-6, CRP, D-dimer, cholesterol and Ox-LDL. In multiple linear regression analysis PAI-1, age, and adiponectin remained statistically associated with t-PA values ($P<0.01$ for all). The weakest significant association ($P=0.046$) was that found between t-PA and D-dimer.

Conclusions: Adiponectin is a main determinant of t-PA level, which may be a good marker of endothelial dysfunction in HD patients.

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2. A QUALIDADE DAS HDL E NÃO TANTO A SUA QUANTIDADE COMO MARCADOR DE RISCO CARDIOVASCULAR NO ACIDENTE VASCULAR CEREBRAL – REALCE PARA A ACTIVIDADE DA PARAOXONASE 1

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A grande combinação de factores de risco por norma presentes no doente que foi vítima de um acidente vascular cerebral (AVC), por natureza um paciente de elevado risco cardiovascular (CV), torna mais difícil a redução da hiperlipidemia. Esta poderá exigir uma intervenção mais eficaz no HDL-c, que continua a ser pouco modificado com os fármacos actuais [1], no sentido de modular a sua actividade ou qualidade [2,3]. A paraoxonase 1 (PON1) é uma enzima constituinte das HDL que tem sido indicada como um dos principais responsáveis pela sua actividade antiaterogénica [4]. Contudo, a forma como se encontra modulada no doente vítima de AVC continua por elucidar. Este trabalho teve como principal objectivo avaliar a possibilidade de usar a qualidade das HDL (dada pelas suas subpopulações e pela actividade PON1) como melhor marcador de risco CV do que os marcadores tradicionais.

Foram incluídos no estudo 32 doentes que tiveram episódio de AVC e 55 controlos. Após consentimento informado, foram recolhidos dados antropo-

métricos e analisados os seguintes parâmetros: glicemia, HbA1c, perfil lipídico (Total-c, TG, LDL-c, HDL-c, Ox-LDL-c e fracções de HDL-c; actividade da PON1 (nmol pnitrophenol/ml/min); perfil inflamatório e angiogénico (PCR, ácido úrico, TNF-α, adiponectina e VEGF) e oxidativo (MDA). Resultados em médias±epm.

Os pacientes com AVC apresentaram um perfil típico de obesidade (IMC e perímetro abdominal aumentados), e dislipidemia (HDL-c reduzido e aumento de Ox-LDL/LDL). Apesar do conteúdo de LDL-c normal, pacientes com AVC mostraram uma percentagem significativamente maior de subpopulações de HDL-pequenas (14,9±1,0%; $p<0,05$) e reduzida de Grandes (33,9±1,8%; $p<0,01$) vs controlo (18,4±1,4 e 41,2±2,2%, respectivamente). A actividade da PON1 estava reduzida no grupo AVC, e associava-se inversamente com as HDL-pequenas e directamente com as HDL-grandes. Os doentes apresentavam ainda valores mais elevados de VEGF e TNF-α e inferiores de adiponectina.

Em conclusão, a funcionalidade ou qualidade das HDL (expressa pela actividade da PON1) e o seu conteúdo específico (subpopulações) poderão vir a ser considerados melhores marcadores de risco cardiometabólico em pacientes vítimas de episódio de AVC do que os parâmetros clássicos de perfil lipídico actualmente em utilização, constituindo-se como ferramentas importantes para melhorar o prognóstico destes doentes de alto risco CV.

Referências: [1] Sharma et al. *Vasc Health Risk Manag*. 2009; [2] Lahoz et al. 2009; [3] Tsompanidi et al. *Atherosclerosis*. 2010; [4] Goswami et al. *Clin Chim Acta.* 2009.

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THROMBOSIS OF THE INFERIOR VENA CAVA IN A YOUNG PATIENT WITH HYPERHOMOCYSTEINEMIA

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Deep venous thrombosis (DVT) is a relatively uncommon condition in patients below 30 years old and its global lifelong incidence in the general population is 0.1%. Venous thrombosis of the inferior vena cava is an even rarer clinical condition, which shares common etiological causes with DVT. Hyperhomocysteinemia is a well-known risk factor for DVT. In this work we present the clinical case of a 24-year old male patient, with past history of vitiligo and hyperthyroidism, who was referred from a peripheral hospital due to a suspected DVT of the inferior part of the left leg while hospitalized to treat a community acquired Pneumonia. Upon admission to the ER, a venous eccopdoppler scan of the inferior limbs was perfomed, which revealed a biiliac/bifemoral DVT, with left side occlusion. To further study the throm-

bus, we performed a thoracic-abdominal-pelvic angio CT scan which showed a partially occlusive thrombosis of the inferior vena cava with extension to the right cardiac atrium, areas of pulmonary infarction and acute pulmonary thromboembolism. The patient remained hospitalized and a therapeutic dosage of enoxaparine was used for hypocoagulation. During hospitalization increased serum levels of homocystein were detected, in the presence of normal serum levels of vitamin B12 and folic acid. Eight months after the initial episode, the patient is under oral hypocoagulation with acenocumarol (INR 2-3) and we registered a progressive mild clinical recovery. The imaging studies recently performed show persistence of the venous thrombosis with partial dissolution of the thrombus, with extension until the intra-hepatic portion of the inferior vena cava.

4. ADIPOCYTOKINE LEVELS IN PORTUGUESE TYPE-2 DIABETES *MELLITUS* PATIENTS ACCORDING TO BODY MASS INDEX

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Diabetes *mellitus* type 2 and obesity are known to be associated. The adipose tissue secretes several adipocytokines, such as adiponectin, leptin and chemerin. Adiponectin has anti-inflammatory activity and protects against metabolic and cardiovascular diseases. Deficiency in leptin has been linked to insulin resistance and vascular dysfunction. Chemerin, a new adipocytokine, apparently associates with inflammation, adipogenesis and lipid and glucose metabolism. Data concerning chemerin levels in pre-diabetics, in lean and obese diabetic, is not consensual. Our aim was to evaluate the adipocytokines levels – adiponectin, leptin and chemerin - in lean, overweight and obese type 2 diabetic patients.

Eighty-three patients (63±10 years old), under oral hypoglycemic therapy, were enrolled in this study, after informed consent. Patients were divided in three groups, according to body mass index (BMI): lean, BMI<24.9 kg/m2 (n=28; 11 females/17 males), overweight, BMI 25.0-29.9 kg/m2 (n=38; 20 females/18 males), and obese, BMI>30.0 kg/m2 (n=17; 10 females/7 males). A control group (n=20) matched for gender and age was also studied. Subjects were evaluated for glucose, glycated hemoglobin, adiponectin, leptin, and chemerin.

The obese group presented significantly lower adiponectin and significantly higher leptin and chemerin values, as compared to the overweight, lean and control groups (for control, the differences remained significant after adjustment for BMI). The overweight, compared to the lean and control pa-

tients, presented significantly lower adiponectin and significantly higher leptin and chemerin levels. The lean group presented significantly higher values than the control (that persisted significant after BMI adjustment). Leptin values differed between male and female, both in patients and controls. Male obese patients presented significantly higher leptin levels than male lean patients, and a trend towards higher values than male controls; male overweight group showed significantly higher leptin levels than lean and control group (that lost significance after BMI adjustment). In female obese patients, leptin values were significantly higher than those of female overweight, lean and control subjects; female overweight showed significantly higher leptin values than female control group that persisted significant after BMI adjustment. Glucose and glycated hemoglobin values did not differ between the 3 groups of diabetics.

In summary, in type 2 diabetic patients, leptin and adiponectin levels seem to be more related with obesity and less with diabetes. Chemerin levels were raised in lean, overweight and obese patients, suggesting that in diabetes type 2, independently of BMI, adipocyte dysfunction occurs. Further studies are needed, but chemerin may be a possible link between obesity and type 2 diabetes *mellitus*.

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6. THE ROLE OF INFLAMMATORY BIOMARKERS IN THE ASSESSMENT OF CORONARY ARTERY DISEASE

DISTINGUIDO COM O"PRÉMIO SPHM-SPACV/BAYER PARA O MELHOR TRABALHO CLÍNICO"

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Acute myocardial infarction (AMI) is a critical clinical presentation of coronary artery disease (CAD) in many asymptomatic patients and often the event is fatal. Establishing the presence of coronary lesions in asymptomatic patients or in symptomatic patients can be a challenging task. Consequently, major clinical research efforts have been dedicated to the identification of patients at higher risk and to the diagnosis of CAD.

The main objective of this study was to investigate several inflammatory markers that may have relevant roles in coronary disease and in the processes involved in plaque rupture as confirmed by the angiographic detection of high-grade luminal obstructions and the artery wall morphology.

Patients with different stages of CAD were included in the study: 60 patients with acute myocardial infarction (AMI) submitted to coronary angiography as reperfusion therapy; and 40 patients with angiographically confirmed CAD suffering from chest discomfort. A group of 60 patients without documented coronary disease as verified by coronary angiography constituted the control (CTR) group. Additionally, a longitudinal study was carried out in the AMI patients at hospital admission before the administration of IIb/IIIa inhibitors and coronary angioplasty, 2 and 40 days after the onset of symptoms.

The results revealed that the circulating levels of ICAM, P-selectin and TNF--α were decreased in CAD patients relative to patients without coronary disease (control group). However the levels of inflammatory biomarkers were increased in acute events and continue to rise in the AMI evolution over 40 days. This trend was not verified for P-selectin that showed a drop at day 2 reflecting the influence of massive anti-platelet therapy measures during angioplasty, and for CRP that rise at day 2. The number of monocytes, neutrophils and lymphocytes were more elevated in CAD patients than in controls. An unambiguous influence of medication in monocytes and neutrophils counts was verified that could not be proved for T lymphocytes or TNF-α, pointing out for the need of alternative therapeutic strategies to modulate these inflammatory responses.

The reported results pointed out the importance of the inflammatory response that remains **after clinical stabilization in AMI patients and that is present in CAD patients, supporting** the concept of a differential response of inflammation in several stages of CAD. Therefore, the work highlighted the complex relationships between the studied biomarkers contributing to a better understand of the pathology and mechanisms of CAD.

Keywords: Inflammatory markers, Coronary lesions, Longitudinal study

7. CYCLOSPORIN-INDUCED NEPHROTOXICITY IS ATTENUATED WITH REPLACEMENT FOR SIROLIMUS – FOCUS ON OXIDATIVE STRESS, INFLAMMATION, PROLIFERATION AND ANGIOGENESIS

DISTINGUIDO COM O"PRÉMIO SPHM-SPACV/BOEHRINGER INGELHEIM PARA O MELHOR TRABALHO EM INVESTIGAÇÃO BÁSICA"

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Sirolimus (SRL) have been pointed as a feasible option for minimize the use of Cyclosporin A (CsA), especially because of putatively less nephrotoxicity. However, the cellular mechanism underlying the renoprotection remains

to be elucidated, and the clinical data is yet insufficient. This study aimed to characterize the histological lesions and the molecular pathways implicated in CsA-induced nephropathy and prevention when converted to SRL.

The following 4 groups (n=6) were tested during 9 wks: Vehicle; CsA (5 mg/kg/day Sandimmun Neoral®); SRL (1 mg/kg/day Rapamune®); Conversion (CsA 3 weeks + SRL 6 weeks). BP and HR were monitored. Blood was collected at 9 week to evaluate: creatinine, BUN, TGs, Total-Chol, glycaemia, glucose tolerance and insulinaemia. Serum levels of inflammatory (IL-2, IL-1β, CRP and TNF-α), proliferative (TGF-β), angiogenesis (VEGF) and lipid peroxidation (MDA assay) markers were assessed. For histological evaluation, kidney was stained in hematoxylin and eosin, periodic Acid of Schiff techniques. Statistics: means±s.e.m., One-way ANOVA and Student's t-test were used ($p<0.05$).

CsA has induced tachycardia, hypertension (146.1±4.0 vs 118.4±2.4; $p<0.001$), hyperglycaemia and kidney lipid peroxidation ($p<0.001$). Moreover, promote important kidney lesions, including glomerular, tubulointerstitial and vascular: mesangial expansion, atrophy, bowman capsule enlargement, hyaline cylinders formation, tubular calcification and vascular congestion, as well as arteriolar vacuolization and arteriolosclerosis. SRL treatment has promoted hyperglycaemia, hypertension (139.5±2.0 vs 118.4±2.4; $p<0.01$), IL-2 ($p<0.001$), TGF-β ($p<0.01$) and VEGF ($p<0.05$) decrease. However, after conversion to SRL, CsA-induced HT and tachycardia were reduced, accompanied by amelioration of kidney dysfunction (normal creatinine and BUN), with reduction of oxidative stress. Moreover, SRL treatment in the conversion group prevented CsA-induced arteriolar vacuolization, glomerular mesangial expansion, hialinosis, atrophy, bowman capsule enlargement, as well as formation of hyaline cylinders. Serum markers reveals, IL-2 serum decrease, followed by IL-6 increase ($p<0.05$) and TGF-β decrease ($p<0.05$).

In conclusion, conversion of CsA to SRL demonstrates cardiorenal benefits, which should be associated with the protective properties of SRL, presumably resulting from the anti-proliferative capacity. These mechanisms deserve better exploitation, namely in clinical practice, in order to fully potentiate their favourable balance efficacy-safety (renoprotection).

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8. MARCADORES DE DOENÇA CARDIOVASCULAR E SUA ASSOCIAÇÃO COM ALTERAÇÕES NA MEMBRANA ERITROCITÁRIA EM UMA POPULAÇÃO PEDIÁTRICA OBESA

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Os glóbulos vermelhos (GV) apresentam mecanismos de defesa limitados, acumulando danos quando expostos a stresses físicos e/ou químicos. Os lípidos da membrana dos GV estão em equilíbrio constante com os lípidos plasmáticos, sendo este o modo pelo qual lípidos lesados são substituídos, reflectindo o balanlo lipídico por períodos mais longos que os lípidos plasmáticos. O eritrócito é, portanto, um bom modelo para estudar os danos nos lípidos e proteínas e o impacto dos hábitos alimentares na composição das membranas celulares. Hábitos alimentares que se encontram normalmente alterados nos obesos. A composição lipídica das membranas influencia as propriedades reológicas e físico-químicas das células, modulando a actividade de transportadores, receptores membranares, … fazendo, assim, variar a sinalização e várias outras funções celulares. O objectivo deste estudo foi analisar o impacto da obesidade no perfil lipídico, insulinorresistência e inflamação, e a ligação dessas alterações com a composição lipídica da membrana do GV.

Foram estudadas 34 crianças e adolescentes obesos [rapazes: 15 (44,1%); idade média: 14,1 anos (8-17)] do Hospital S. João e do Hospital Infantil Maria Pia, Porto. O grupo foi dividido segundo os percentis de IMC (gráficos de crescimento do CDC (2000) ajustados para a idade e sexo) em: obesos (n=17): IMC ≥ percentil 95, sobrepeso (n=8): percentil de IMC ≥ 85 e < 95; controlos (n=9): IMC < percentil 85. Os três grupos estavam ajustados para idade, sexo e estadío de Tanner. Foi determinado os níveis plasmáticos de triglicerídeos, colesterol, HDL-colesterol, LDL-colesterol, lipoproteína (a), apolipoproteína A e B, proteína C-reativa, glicose e insulina. Um estudo hematológico básico foi realizado. Foram estudados marcadores membranares de lesão eritrocitária: hemoglobina ligada a membrana, carbonilação proteica, peroxidação lipídica e perfil de banda 3. O perfil de ácidos gordos da membrana foi também determinado.

Os indivíduos obesos apresentaram aumento geral dos marcadores de risco de doença cardiovascular (DCV), quando comparados com os controlos, apresentando um perfil lipídico mais aterogénico, aumento da resistência à insulina e da inflamação. Nenhuma diferença foi encontrada no eritrograma ou nos marcadores de lesão eritrocitária. Relativamente ao perfil de AG, uma proporção crescente dos AG 20:0, 18:3n3, 20:3n6 e 22:4n6 foram encontrados para indivíduos com sobrepeso e obesos, em relação aos controlos. Estes mesmos AG apresentaram igualmente associações significativas com o aumento dos marcadores de DCV estudados.

Mais estudos são necessários para esclarecer o modo como alterações do perfil de AG da membrana eritrocitária se relacionam com marcadores de risco de DCV.

PARTICIPAÇÃO EM REUNIÕES CIENTÍFICAS E CONGRESSOS INTERNACIONAIS

XXIIND INTERNATIONAL FIBRINOGEN WORKSHOP

Decorreu em Brighton de 4 a 6 de Julho o *XXIInd International Fibrinogen Workshop organizado pelo Leeds Institute for Genetics, Health and Therapeutics*.

A SPHM esteve representada pela presidente, que apresentou o trabalho "CD47 agonist peptide effects on human erythrocyte nitric oxide mobilization in presence of fibrinogen"

CD47 AGONIST PEPTIDE EFFECTS ON HUMAN ERYTHROCYTE NITRC OXIDE MOBILIZATION IN PRESENCE OF FIBRINOGEN

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Fibrinogen is a plasma protein with functions, in haemostasis, cell adhesion and inflammation. It behaves as an acute phase protein and as a hemorheological factor by promoting the formation of erythrocyte aggregates. The erythrocyte hyperaggregation state induced by fibrinogen takes place in various metabolic and cardiovascular diseases such as diabetes, arterial hypertension and atherosclerosis.

Soluble form of fibrinogen binds to erythrocyte CD47 and at hiperfibrinogenemia modulates nitric oxide metabolism in dependence of band 3 phosphorylation degree. Soluble thrombomodulin is an inflammatory marker that binds erythrocyte CD47 in a sequence peptide known as 4N1K.

The aim of this work was to study the influence of the CD47 agonist peptide, 4N1K, on the erythrocyte nitric oxide (NO) metabolism in absence and under the presence of high fibrinogen concentration.

In this *in-vitro* study, whole blood samples were harvested from healthy subjects and NO, peroxynitrite, nitrite, nitrate and S-nitroglutathione (GSNO) were determined in presence of 4N1K and also under high fibrinogen concentrations. The results obtained, when 4N1K is present in absence and in

presence of fibrinogen show, in relation to control, (1) no variations on the levels the erythrocyte NO efflux; (2) increased concentrations of the reactive nitrogen species namely peroxynitrite *($p<0.05$; $p<0.005$)*, nitrite ($p<0.0001$; *$p<0.001$*) and nitrate ($p<0.0001$;*$p<0.001$*);

(3) increased GSNO concentrations ($p<0.001$; *$p<0,001$*). At variance no changes were observed in GSNO levels in presence of only high fibrinogen concentrations.

In conclusion the CD47 agonist peptide 4N1K induces erythrocyte NO mobilization similar to that observed for high fibrinogen concentrations. Under inflammatory stimulus, *in vitro*, erythrocyte reactive nitrogen species change its concentrations.

4TH EUROSUMMER SCHOOL ON BIORHEOLOGY & SYMPOSIUM ON MICRO AND NANO MECHANICS AND MECHANOBIOLOGY OF CELLS, TISSUES AND SYSTEMS

Decorreu em Varna de 29 de Agosto a 2 de Setembro o *4th Eurosummer School on Biorheology & Symposium on Micro and Nano Mechanics and Mechanobiology of Cells, Tissues and Systems.*

A reunião foi organizada pela Bulgarian Society of Biorheology em cooperação com o Institute of Mechanics and Biomechanics to the Bulgarian Academy of Sciences e a European Society for Clinical Hemorheology and Microcirculation (E.S.C.H.M.) A presidente da SPHM,além de integrar a International Advisory Committee, foi convidada a proferir uma lição intitulada "Nitric oxide as a hemorheological factor". Também moderou uma sessão de comunicações sobre o tema "Hemorheologicl disturbances in experimental animals"

NITRIC OXIDE AS A HEMORHEOLOGICAL FACTOR

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Blood viscosity (BV) depends on plasma viscosity, hematocrit, erythrocyte aggregation (EA), erythrocyte deformability (ED) and fibrinogen values. Impair ED ability is influent in blood viscoeleasticity values at both high and low shear rates while either enhanced or diminished EA tendencies are determinant in low shear rate decreasing their influence in high shear rates.

Erythrocyte deformability is a complex hemorheological parameter that depends on the surface-volume ratio, media globular haemoglobin concentration, membrane lipid fluidity and cytoskeleton proteins phosphorylation degree. RBCs protein kinase C (PKC) is an second messenger that influences the protein tyrosine kinase (PTK) and protein tyrosine phosphates (PTP)

enzymes activities. PTK and PTP are implicated in protein band 3 phosphorylation degrees and are both influenced by peroxynitrite levels.

Nitric oxide (NO) produced by endothelial cells, known as a vasodilator in physiological conditions interacts with RBCs via protein band 3, being scavenged by either haemoglobin originating S-nitrosohemoglobin or nitrosylhemoglobin and by glutathione forming S- nitrosoglutathione (GSNO). Beyond the NO preservation inside the erythrocytes derivative molecules such as nitrite, nitrate and peroxynitrites are also present in RBCs. The peroxynitrites levels are a consequence of the nitrogen and oxygen oxidative stress status.

RBCs membrane protein band 3 when phosphorylated favours NO efflux without ED changes. Binding of acetylcholine (ACh) to RBCs membrane acetylcholinesterase (AChE) originate a signal transduction mechanism involving protein Gi and protein band 3 that stimulates NO efflux and ED.

RBCs receive NO from spermine- NONOate with RBCs deformability improvement.

Among the hemostatic and the inflammatory functions attributed to plasma fibrinogen it is also an hemorheological parameter that influences plasma viscosity, contributes for the ability of red blood cell (RBCs) to aggregate with repercussion on BV. Fibrinogen binds to CD47 erythrocyte membrane decreases the RBCs NO efflux and enhances the GSNO formation. Fibrinogen preserves the erythrocyte NO scavenger property letting ED unchanged.

However RBCs in presence of high fibrinogen concentrations and (i) when protein band 3 is dephosphorylated ED increases at low shear rate without NO efflux modifications (ii) when protein band 3 is phosphorylated the NO efflux increased and EEI maintained the normal level.

Sepsis patients have increase levels of nitrosothiols that act as NO donors and decrease ED. Hypercholerolemia, hypertension and erectile dysfunction are vascular dysfunction pathologies with impaired ED and RBCs ability to liberate NO when tested in vitro.

Erythrocyte NO efflux is negatively associated with carotid intima-media thickness and independently associated with early stages of atherosclerosis in patients with lupus erythematosus.

ARTIGOS PUBLICADOS NO ESTRANGEIRO

DIFFERENTIAL EFFECT OF SOLUBLE FIBRINOGEN AS A NEUTROPHIL ACTIVATOR.

de Almeida VV, Calado A, Rosário HS, Saldanha C.
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Abstract

A fundamental paradigm involved in acute inflammatory responses to invading pathogens and tissue damage is the migration of specific leukocyte populations to the affected tissues to mount an initial innate response to the aggression. The recruitment of polymorphonuclear neutrophils (PMNs) from the blood is a central event in this respect. The aim of this study was to understand whether fibrinogen is able to modulate the pattern of neutrophil activation and thus contribute to neutrophil recruitment. We demonstrated that fibrinogen induces free radical production by neutrophils without modifying the activation status of Mac-1 (αMβ2, CD11b/CD18), the previously identified neutrophil receptor for fibrinogen. This data indicates that fibrinogen must have an additional different binding site in the neutrophil membrane. Importantly, we propose that as Mac-1 activation was not affected by the binding of fibrinogen, activated neutrophils can further maintain their ability to marginate, roll and adhere to the endothelial walls [**Clin Hemorheol Microcirc. 2012 Jan 1;51(1):1-20].**

ERYTHROCYTE AS A BIOLOGICAL SENSOR.

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Abstract

The erythrocytes ability of sensing the local oxygen gradient through the hemoglobin conformation, along with changes in nitric oxide mobilization and

vasomotor repercussions at the microcirculation, were reviewed in detail in this article. Different approachs trying to explain the erythrocyte death were additionally documented. Also, the influence of several types of molecules (vasoactive, oxidant/reductor) on the erythrocyte roles as sensor of (i) oxygen tissue needs, (ii) blood viscosity and myogenic environment, (iii) and inflammatory conditions were mentioned in order to highlight its physiologycal function and substitute the erroneous idea of the erythrocyte being simply a hemoglobin sac content **[Clin Hemorheol Microcirc. 2012 Jan 1;51(2):129-37].**

CELL-SPECIFIC REGULATION OF ACETYLCHOLINESTERASE EXPRESSION UNDER INFLAMMATORY CONDITIONS.

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Abstract

Acetylcholine (ACh) has been shown to exert an anti-inflammatory function by down-modulating the expression of pro-inflammatory cytokines. Its availability can be regulated at different levels, namely at its synthesis and degradation steps. Accordingly, the expression of acetylcholinesterase (AChE), the enzyme responsible for ACh hydrolysis, has been observed to be modulated in inflammation. To further address the mechanisms underlying this effect, we aimed here at characterizing AChE expression in distinct cellular types pivotal to the inflammatory response. This study was performed in the human acute leukaemia monocytyc cell line, THP-1, in human monocyte-derived primary macrophages and in human umbilical cord vein endothelial cells (HUVEC). In order to subject these cells to inflammatory conditions, THP-1 and macrophage were treated with lipopolysaccharide (LPS) from E.coli and HUVEC were stimulated with the tumour necrosis factor α (TNF-α). Our results showed that although AChE expression was generally up-regulated at the mRNA level under inflammatory conditions, distinct AChE protein expression profiles were surprisingly observed among the distinct cellular types studied. Altogether, these results argue for the existence of cell specific mechanisms that regulate the expression of acetylcholinesterase in inflammation **[Clin Hemorheol Microcirc. 2012;50(3):213-9].**

ERYTHROCYTE DEFORMABILITY DEPENDENCE ON BAND 3 PROTEIN IN AN IN-VITRO MODEL OF HYPERFIBRINOGENEMIA.

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Abstract

Recent evidence has shown that plasma fibrinogen, a major cardiovascular risk factor, interacts with the erythrocyte membrane and acts to influence blood flow via erythrocyte nitric oxide (NO) modulation. In the present in--vitro study, whole blood samples were harvested from healthy subjects and aliquots were incubated in the absence (control aliquots) and presence of fibrinogen at different degrees of band 3 phosphorylation, and the erythrocyte deformability was determined. The present study shows that in the presence of higher fibrinogen concentrations, similar to those found in inflammatory conditions, erythrocyte deformability is increased only when band 3 is dephosphorylated by the presence of syk inhibitor and at low shear stress. On the contrary, no changes were verified in the presence of fibrinogen when band 3 is allowed to be phosphorylated by inhibiting the phosphotyrosine phosphatase enzyme activity with calpeptin. We also observed that the presence of fibrinogen at higher concentration does not induce changes in erythrocyte deformability in the absence of modulators of the band 3 phosphorylation degree. However, the mechanisms by which fibrinogen signalling modulates erythrocyte function remain to be clarified and are currently under study **[Clin Hemorheol Microcirc. 2011;49(1-4):463-72].**

ERYTHROCYTE AS A LINK BETWEEN BASIC AND CLINICAL RESEARCH.

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Source

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Abstract

We review the major hemorheological experimental studies that show the erythrocyte aggregation as a link between basic and clinical research. The results of the clinical cross-sectional and longitudinal studies presented here will highlight the possible association between erythrocyte aggregation and plasma fibrinogen. Basic studies conducted in vitro are also mentioned as for its relevance in answering questions raised in clinical settings, as well as and in understanding the underlying influent factors in the erythrocyte tendency to aggregate and disaggregate [**Clin Hemorheol Microcirc. 2011;49(1-4):407-16**].

EVIDENCE THAT THE DEGREE OF BAND 3 PHOSPHORYLATION MODULATES HUMAN ERYTHROCYTES NITRIC OXIDE EFFLUX–IN VITRO MODEL OF HYPERFIBRINOGENEMIA.

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Abstract

Recent evidence has shown that plasma fibrinogen, a major cardiovascular risk factor, interacts with the erythrocyte membrane and acts to influence blood flow via erythrocyte nitric oxide (NO) modulation. In the present pioneer in-vitro study, whole blood samples were harvested from healthy subjects and aliquots were incubated in the absence (control aliquots) and presence of fibrinogen at different degrees of band 3 phosphorylation, and the levels of NO, nitrite, nitrate and S-nitroglutathione (GSNO) were determined. Hyperfibrinogenemia interferes with erythrocyte NO mobilization without changing its efflux in a way that seems to be dependent of the degree of band 3 phosphorylation. In presence of higher fibrinogen concentrations the NO efflux is reinforced when band 3 is phosphorylated ($p < 0.001$). Higher levels of nitrite, nitrate and GSNO were documented ($p < 0.05$). However, the mechanisms by which fibrinogen signalling modulates erythrocyte function remain to be clarified and are currently under study. These conditions may be considered an approach to be followed in blood storage for transfusions **[Biochim Biophys Acta. 2012 Mar;1818(3):481-90]**

INTEGRIN-ASSOCIATED PROTEIN (CD47) IS A PUTATIVE MEDIATOR FOR SOLUBLE FIBRINOGEN INTERACTION WITH HUMAN RED BLOOD CELLS MEMBRANE.

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Abstract

Fibrinogen is a multifunctional plasma protein that plays a crucial role in several biological processes. Elevated fibrinogen induces erythrocyte hyperaggregation, suggesting an interaction between this protein and red blood cells (RBCs). Several studies support the concept that fibrinogen interacts with RBC membrane and this binding, due to specific and non-specific mechanisms, may be a trigger to RBC hyperaggregation in inflammation. The main goals of our work were to prove that human RBCs are able to specifically bind soluble fibrinogen, and identify membrane molecular targets that could be involved in this process. RBCs were first isolated from blood of healthy individuals and then separated in different age fractions by discontinuous Percoll gradients. After isolation RBC samples were incubated with human soluble fibrinogen and/or with a blocking antibody against CD47 followed by fluorescence confocal microscopy, flow cytometry acquisitions and zeta potential measurements. Our data show that soluble fibrinogen interacts with the human RBC membrane in an age-dependent manner, with younger RBCs interacting more with soluble fibrinogen than the older cells. Importantly, this interaction is abrogated in the presence of a specific antibody against CD47. Our results support a specific and age-dependent interaction of soluble fibrinogen with human RBC membrane; additionally we present CD47 as a pu-

tative mediator in this process. This interaction may contribute to RBC hyperaggregation in inflammation **[Biochem Res Int. 2012;2012:261736].**

BEHAVIOUR OF HUMAN ERYTHROCYTE AGGREGATION IN PRESENCE OF AUTOLOGOUS LIPOPROTEINS.

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Abstract

The aim of this work was to evaluate in vitro the effect of autologous plasma lipoprotein subfractions on erythrocyte tendency to aggregate. Aliquots of human blood samples were enriched or not (control) with their own HDL-C, LDL-C, or VLDL-C fractions obtained from the same batch by density gradient ultracentrifugation. Plasma osmolality and erythrocyte aggregation index (EAI) were determined. Blood aliquots enriched with LDL--C and HDL-C showed significant higher EAI than untreated aliquots, whereas enrichment with VLDL-C does not induce significant EAI changes. For the same range of lipoprotein concentrations expressed as percentage of osmolality variation, the EAI variation was positive and higher in presence of HDL-C than upon enrichment with LDL-C ($P < 0.01$). Particle size, up to LDL diameter values, seems to reinforce erythrocyte tendency to aggregate at the same plasma osmolality (particle number) range of values **[Atherosclerosis. 2011 Dec;219(2):821-6].**

HEMORHEOLOGICAL PARAMETERS ARE RELATED TO SUBCLINICAL ATHEROSCLEROSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS PATIENTS.

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Abstract

Objectives**:** Rheological characteristics of blood are strongly linked to atherothrombosis in the general population, but its contribution to atherosclerosis in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) is currently unclear. This work examines the relationship between blood rheology, traditional cardiovascular (CV) risk factors, inflammation and subclinical atherosclerosis in SLE and RA.

Methods: Whole blood viscosity (WBV), plasma viscosity (PV), erythrocyte deformability (ED), aggregation (EA) and erythrocyte NO production

were measured in 197 patients (96 SLE and 101 RA) and compared to 97 controls, all females without previous CV events. Clinical information was obtained and fasting lipids and acute phase reactants were measured. The relationship between hemorheological parameters, CV risk factors and inflammation was assessed in patients and the impact of these variables on carotid intima-media thickness (cIMT) was evaluated in univariate followed by multivariate regression analyses.

Results**:** WBV and ED are significantly lower in patients, while EA is elevated as compared with controls. Hemorheological disturbances correlate with CV risk factors and markers of inflammation and are more profound in patients with metabolic syndrome. Multivariable analysis showed that menopause (OR 34.72, 95%CI 4.44-271.77), obesity (OR 4.09, 95%CI 1.00-16.68) and WBV (OR 3.98; 95%CI 1.23-12.83) are positively associated whereas current corticosteroid dose (OR 0.87; 95%CI 0.78-0.98), and erythrocyte NO production (OR 0.16; 95%CI 0.05-0.52) are negatively associated with cIMT.

Conclusion**:** Disturbed hemorheological parameters in SLE and RA women are related to the presence of CV risk factors and inflammation. WBV and erythrocyte NO are independently associated with the early stages of atherosclerosis **[Atherosclerosis. 2011 Dec;219(2):821-6].**