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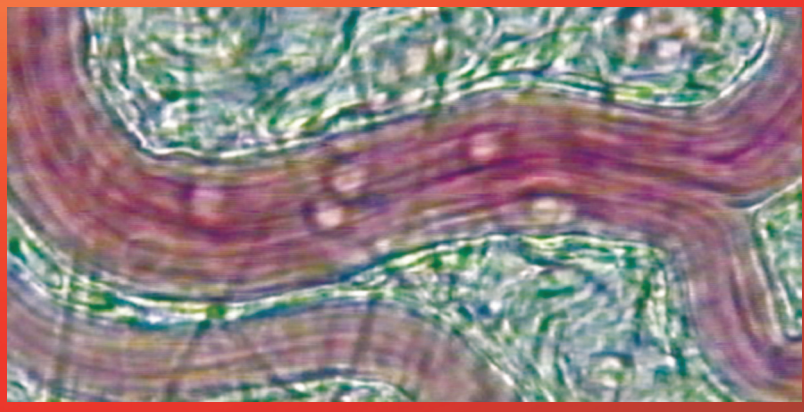

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BOLETIM

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Sumário / Summary

Sociedade Portuguesa de Hemorreologia e Microcirculação

Presidente Honorário: Prof. Doutor João Alcindo Martins e Silva

Referência da capa: Vénula pós-capilar (diâmetro aproximado: 30 mm) de rede microvascular em mesentério de rato (*Rattus norvegicus*), observada por microscopia intravital de transiluminação. No interior do vaso sanguíneo visualizam-se leucócitos a interagir com a parede vascular. Imagem obtida por Henrique Sobral do Rosário (Instituto de Biopatologia Química – Prof.ª Doutora Carlota Saldanha, Faculdade de Medicina de Lisboa; Unidade de Biopatologia Vascular, Instituto de Medicina Molecular)

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FIBRINOGÉNIO E MONÓXIDO DE AZOTO

Neste número do BSPH, as notícias sobre as duas reuniões internacionais em que a nossa sociedade esteve representada pode suscitar alguma curiosidade sobre a ligação subjacente aos títulos e conteúdos dos temas das duas apresentações feitas. A ligação ou relação entre fibrinogénio e o monóxido de azoto vai para além do eritrócito. Em editorais anteriores temos escrito sobre a interligação funcional das duas moléculas e sobre as respectivas propriedades hemorrelógicas e inflamatórias

Um dos primeiros trabalhos "in vivo" realizado com ratos demonstrou que a administração de um inibidor da sintase endotelial do monóxido de azoto (eNOS) aumentava a concentração plasmática do fibrinogénio, sugerindo a instalação de uma resposta inflamatória. Posteriormente verificou-se que, prolongando o tempo de infusão do inibidor da eNOS, os níveis circulantes da proteína de fase aguda normalizavam. Haveria um mecanismo sem associação aparente aos receptores da angiotensina -II responsável pelo retorno ao valor basal. Outros trabalhos têm demonstrado que a angiotensina potencia a entrada de fibrinogénio para a parede do vaso e ou para a placa de ateroma. Está por demonstrar se será esta a explicação para o desaparecimento da hiperfibrinogenemia induzida por inibição da eNOS. Enquanto no endotélio íntegro o NO induz vasodilatação, o contrário acontece no endotélio disfuncional. Arteríolas de cremáster de rato quando perfundidas com fibrinogénio contraiem-se, e o grupo de Lominadze sugere que o mecanismo explicativo da vasoconstrição esteja interligado ao da inibição da eNOS. Verificaram os autores que a ligação do fibrinogénio ao endotélio ocorre pela molécula de adesão intercelular (ICAM-1) que sinaliza a via de transdução de sinal da eNOS, com inibição desta enzima.

Há evidência epidemiológica de que a concentração plasmática do fibrinogénio, acima do normal, constituiu um factor independente preditivo de risco de doença cardiovascular. O stress oxidativo e nitroso estão presentes nas doenças vasculares, e o estudo efectuado pela *Cleveland Clinical Foundation* demonstrou que a nitração das moléculas de fibrinogénio é considerada um factor de risco para a doença arterial coronária. A explicação reside na arquitectura da rede de fibrina formada, nos doentes com patologia arterial coronária, que tem propriedades pró-trombóticas. Nesta condição, a rede de fibrina é mais quebradiça ao stress mecânico, o que aumenta a probabilidade de risco de formação de micro êmbolos.

Há sempre duas "faces "funcionais nas moléculas, como acima mencionávamos aquando da vaso actividade do NO. Na displasia broncopulmonar, a inalação de NO é uma

terapêutica de sucesso que decresce a deposição de fibrina e a diminuição da síntese de interleucinas pró-inflamatórias, melhorando a componente fibrinolítica.

O fibrinogénio e o NO participam, interligam-se nos macro, micro e nano processos da inflamação, da hemostase e da hemorreologia.

Foi apenas uma aragem breve da época de férias, bom recomeço

Carlota Saldanha
Presidente da SPHM

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HEPARIN-INDUCED THROMBOYTOPENIA: A MISDIAGNOSED CLINICAL SYNDROME.

Anna Vittoria Mattioli[1]

ABSTRACT

Heparin-induced thrombocytopenia (HIT) is an immune adverse drug-induced reaction characterized by thrombocytopenia and an increased incidence of thrombosis following subsequent exposure to heparin. Clinical pictures included thrombocytopenia followed by venous thrombosis or arterial thrombosis, the most frequent clinical presentations are deep venous thrombosis and pulmonary embolism. The pathophysiology of HIT is complex, involving the activation of coagulation, endothelial dysfunction, and platelet activation. HIT is induced by heparin dependent IgG antibodies that activate platelets. Clinical suspicion f HIT can be confirm by using two different types of laboratory assay: a platelet activation assays and immunoassays for detection of PF4--heparin antibodies.

Recent 2012 practice guidelines from the ACCP recommended discontinuation of heparin and administration of direct thrombin inhibitors and factor Xa inhibitors.

INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is an immune-mediated adverse drug reaction that occurs following exposure to unfractionated heparin (UFH) or low-molecular--weight heparin (LMWH) (1,2). Heparin is among the most frequently prescribed medications in cardiovascular disease and in surgery as prevention of deep vein thrombosis, with million patients treated annually. HIT is an important adverse drug reaction to heparin. HIT occurs in approximately 0.5–5% of patients treated with heparin and up to 24% in cardiac surgery patients (3,4).

Patients with cardiovascular disease are at particular risk for the development of HIT antibodies. As many as 25% to 50% of patients who undergo cardiac surgery develop positive levels of anti heparin/PF4 antibodies postoperatively (5).

It has been reported to HIT is an immuno-mediated syndrome due to IgG antibodies against platelets factor-4/heparin complex that activates platelets by way of their FccIIa receptors (6). During UFH infusion,

[1] Department of Life Science, University of Modena and Reggio Emilia (Italy)
Address for correspondence:Anna Vittoria Mattioli, MD PhD, FACC, FESC. Department of Life Science – University of Modena and R.E.,Via del pozzo, 71 41100 Modena (Italy).
Phone: +39 59 4224043 Fax: +39 59 2055426 E-mail: annavittoria.mattioli@unimore.it

PF4 levels increase 15- to 30-fold for several hours, by displacing PF4 from endothelial cell surfaces. PF4/heparin complexes bind to platelet surfaces. IgG antibodies recognize neoepitope sites on PF4, leading to formation of PF4/heparin/IgG complexes on the platelet surface. This phenomenon leads to platelet activation. Activated platelets release additional PF4 that induces a cycle of progressive platelet and coagulation activation. Binding of HIT-inducing antibodies to a complex of heparin and platelet factor 4 (PF4) produces platelet activation and aggregation, including formation of procoagulant, platelet-derived microparticles as well as endothelium activation leading to thrombus formation in either the venous or arterial system. (7,8).

CLINICAL FEATURES

Heparin-induced thrombocytopenia type II (HIT) is clinically considered when the platelet count falls by 50% or more of the baseline value (thrombocytopenia), occurring temporally between day 5 and 14 of therapy, and usually followed by fatal paradoxical thrombotic events.

The timing of thrombocytopenia is influenced by the presence or absence of prior exposure to UFH. In patients who have been exposed to UFH within the previous 3 weeks, the thrombocytopenia begins at a median time of 10.5 hours after the initiation of UFH therapy. These patients already have circulating heparin-dependent antibodies that developed during the prior treatment with UFH.

In rare circumstances, HIT may begin several days after heparin has already been stopped (***delayed-onset heparin-induced thrombocytopenia***) and this is associated with strongly positive tests for HIT antibodies. Delay onset HIT is very dangerous because it is always undiagnosed due to the delay in clinical manifestation. Delay onset HIT can cause devastating venous thromboembolism or arterial clots, prolongs hospitalization, and increases costs. (9)

At least four factors influence the frequency of HIT: type of heparin, duration of heparin treatment, patient population, and gender. The risk of HIT is higher in patients treated with unfractionated heparin compared with patients treated with low molecular weight heparin and to patients treated with fondaparinux. Female are more likely to develop HIT antibodies during prophylaxis with heparin. Highest reported frequencies of HIT are in postsurgical thromboprophylaxis compared to medical treatment. (10,11) Patients at highest risk for HIT/thrombosis are critically ill patients and post cardiovascular surgery patients. A high number of cardiac surgery patients (19%) has already develop antibodies before surgery. Most of these patients has a history of prior exposure to UFH, and the prevalence of antibodies detected after cardiac surgery in this heavily treated population is higher compared with patients who have no prior exposure to unfractionated heparin (83% on the fifth day of treatment). These results suggest a mechanism of anamnestic response (2).

Data from the CATCH study shows a higher incidence of throm-

bocytopenia among patients being treated with UFH or LMWH than previously reported (12). Patients who develop thrombocytopenia has a lower baseline platelet count and a lower body mass index, and also are more likely to be admitted because of cardiovascular diseases: i.e. acute coronary syndrome or cardiovascular surgery. The CATCH investigators also find a direct and consistent relationship between the type, route, and duration of heparin therapy and the likelihood of thrombocytopenia.

Patients that receive UFH intravenously are at higher risk than those who receive UFH or LMWH subcutaneously.

The risk increases with longer heparin exposure, 4% per 1-day increment beyond 4 days of heparin therapy. This observation has important implications for routine clinical practice.

The median platelet count nadir in HIT is about 60 x 10^9/L. Most patients show a 50% or greater decrease in the platelet count. In postoperative patients, the appropriate ‘‘baseline’’ platelet count is not the preoperative platelet count, but rather the highest postoperative platelet count preceding the HIT-associated platelet count decrease (13,14).

After adjustment for important covariates, the CATCH study found that thrombocytopenia, in particular, a greater than 70% reduction in platelet count from baseline, remained independently associated with adverse short-term clinical outcomes.

Clinically, the majority of patients who develop HIT antibodies do not develop thrombocytopenia and thrombosis. In addition, there are several potential explanations for thrombocytopenia in patients receiving heparin. Although there are sensitive assays available to detect HIT antibodies, in clinical practice, test results are not always available in a timely fashion. Moreover, the tests often detect non-pathogenic antibodies inducing diagnostic doubt. For these reasons, in evaluating a patient for possible HIT, a clinical scoring system can help (15). The most frequently used is the 4 Ts Score, evaluates Thrombocytopenia, its Timing, the presence of Thrombosis (or other sequelae of HIT), and whether oTher plausible. The 4Ts Score has a high--negative predictive value; a low score (<3 points) makes HIT unlikely (< 2%). However the positive predictive value varies in different clinical settings. In some settings, a high score predicts a high likelihood of HIT.

Thrombosis is the main contributor to morbidity and mortality associated with HIT, and HIT is fatal in 5–10 % of patients, due to thrombotic events. Thrombosis can occur in any vascular bed, however venous thrombosis is more common than arterial thrombosis and the most frequent clinical presentations are deep venous thrombosis and pulmonary embolism.

LABORATORY TESTS

Two general types of laboratory assay are used to confirm the diagnosis: platelet activation assays such as the serotonin release assay (SRA) and immunoassays such as the enzyme-linked immunosorbent assay

(ELISA) for detection of PF4-heparin antibodies.

Activation assays, such as the platelet serotonin release assay, detect HIT-IgG on the basis of their ability to activate platelets. The SRA is considered to be the gold standard for a laboratory diagnosis of HIT. The SRA measures the platelet-activation response to the anti–PF4- heparin complex as opposed to solely determining the presence of antibody (16,17)

Commercial antigen assays, the enzyme-linked immunosorbent assay (ELISA) for detection of PF4-heparin antibodies is easier to find in clinical practice (18,19).

Commercially available ELISAs are highly sensitive in the detection of PF4-heparin–Ig G antibodies; however, nonpathologic, non– platelet--activating antibodies are also detected by these assays. As such, available polyspecific ELISAs have low specificity for PF4-heparin platelet--activating antibodies.

However, because HIT antibodies can be transient, it is important the timing for serum or plasma tests (20,21). Anti heparin/PF4 antibodies persist for a relatively long period, and this persistence is

associated with a high risk of HIT and HIT-thrombosis (20). Patients in whom heparin/PF4 antibodies are already detectable before surgery as a result of previous exposure to heparin has an even greater increase of titer after surgery and a more prolonged persistence of positivity during follow-up. These patients are also more likely to have thrombotic events during follow-up (22).

DELAYED-ONSET HEPARIN--INDUCED THROMBOCYTOPENIA

Delayed-onset HIT is a rare, often--unrecognized form of HIT. Few case reports describe this syndrome. Delayed-onset HIT was first described by Warkentin and Kelton, and included thrombocytopenia and thrombosis at least 5 days after heparin cessation (9). The 5-day period was arbitrarily chosen to impress that clinical sequelae occur after circulating heparin is eliminated.

The authors describe 12 patients who presented an average of 9.2 days (range 5 to 19 days) after heparin cessation. As a result of lack of disease recognition, 9 patients received additional heparin, resulting in a further decrease in platelet count and thrombosis complications.

Shortly thereafter, Rice et al reported a series of 14 patients with delayed-onset HIT. The criteria, however, for recognition of delayed-onset HIT differed. Rice et al required heparin exposure, discharge after a reasonably benign hospital course during which HIT went unrecognized, objectively proven venous or arterial thromboembolism, and thrombocytopenia at an appropriate time after heparin reexposure.

The different descriptions of delayed-onset heparin-induced thrombocytopenia vary in 2 important areas. The definition by Rice is more consistent with delayed recognition of HIT because heparin--induced thrombocytopenia could have presented clinically during the first heparin exposure but gone unrecognized.

According to Warkentin and Kelton, delayed-onset (as the name suggests) implies that the clinical situation becomes evident days after heparin therapy is complete. Warkentin and Kelton require a platelet count demonstrating thrombocytopenia at presentation, whereas Rice et al. accept thrombocytopenia after heparin reexposure (9).

Both Warkentin and Kelton and Rice et al demonstrate that heparin complications can become evident well after the initial heparin exposure is complete and the heparin is withdrawn (23).

We find that antibodies persist in patients for a long period after cessation of heparin therapy (median time to a negative antigen assay of 90 days); we also observe that in a number of patients antibodies persisted for many months after exposure, similarly to what happen to antibodies induced by other drugs i.e. sulfonamides. Importantly, the number of thrombotic events decreases over time in patients with persisting antibodies, but there remained a higher long-term risk of events. The presence of circulating antibodies is one of the possible mechanisms invoked to explain delay-onset HIT (20).

THERAPY

Recent 2012 practice guidelines from the ACCP for the treatment of HIT include recommendations for platelet count monitoring for patients with a minimum heparin exposure of at least 4 days (24).

The first step in treatment of HIT is discontinuation of all heparin products, including heparin flushes and heparin-coated catheters. In addition to heparin cessation, appropriate non-heparin anticoagulants should be started immediately, even if in the absence of thrombosis.

Current treatment is focused on reduction of thrombin generation via direct thrombin inhibition (e.g., bivalirudin, argatroban, lepirudin,) or indirect factor Xa inhibition (e.g., fondaparinux or danaparoid) (24). Treatment with direct thrombin inhibitors is strongly recommended in patients with HIT. While both direct thrombin inhibitors (DTIs) and factor Xa inhibitors have been used to treat patients with HIT, few data on randomized trials are available so that guidelines focused on historical control studies.

For patients with HITT, ACCP guidelines recommend use of argatroban, lepirudin and danaparoid (no longer available in the U.S.) over continuation of heparin products or use of vitamin K antagonist therapy (Grade 1C) or other non-heparin anticoagulants (Grade 2C). (24) The ACCP guidelines for treatment of HIT also include recommendations for use of platelet transfusions.

These guidelines are easily applicable to regular HIT but the late diagnosis of delay-onset HIT could cause a prolongation of heparin treatment with life-threatening complications.

With the availability of nonheparin anticoagulants, the potential risk of reexposing previous heparin-induced thrombocytopenia patients to long courses of heparin appears unwarranted.

In conclusion, HIT is a serious adverse drug reaction with potentially fatal consequences. Due to wide varia-

bility in the clinical presentation and limitations of laboratory testing, the diagnosis can be difficult. Treatment of HIT and of thrombosis associated with HIT can also be difficult (25).

Despite the availability of several non-heparin anticoagulant therapeutic options, very little quality data support the use of these agents in patients with HIT (25).

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CONCEITOS SOBRE HEMORREOLOGIA E MICROCIRCULAÇÃO HUMANAS.

J. Martins e Silva[1]

TEMA 4 – MICROCIRCULAÇÃO: ESTRUTURA E FUNÇÕES PRINCIPAIS

Estrutura – A *microcirculação* é o segmento da rede vascular que perfunde os tecidos corporais, constituída pelos vasos mais estreitos da circulação (arteríolas, capilares e vénulas). Por via das anteriores características, a microcirculação contrasta com a *macrocirculação*, a qual veicula o sangue entre os diversos órgãos através de artérias, veias e anastomoses arteriovenosas. Em condições fisiológicas, o sangue proveniente das artérias flui para as arteríolas (ramificações finais do sistema arterial), donde passa aos capilares e, destes, para as vénulas (segmentos iniciais do sistema venoso), que desembocam nas veias de retorno, até ao coração (Fig. 2).

As arteríolas (diâmetro: 10-100 μm), apesar de conterem somente uma a duas camadas de músculo liso, são estruturas com abundante inervação pelo sistema simpático, de que resulta serem estes vasos os principais determinantes da *resistência vascular periférica*, na dependência de receptores pós-junccionais α_1 e α_2. Explica-se assim que a maior variação nos valores da pressão sanguínea e da velocidade de perfusão sanguínea ocorra exactamente na transição das arteríolas para os capilares. Algumas das arteríolas (com cerca de 8 μm de diâmetro médio) que transportam o sangue directamente para as vénulas sem que que este passe pela rede capilar, são designados por *meta-arteríolas* ou *arteríolas terminais*. Estas anastomoses arterio-venosas estão igualmente sob o controlo do simpático. Não participam nas trocas de gases, nutrientes ou produtos metabólicos.

As vénulas pós-capilares (diâmetro: 10-50 μm) estão limitadas ao en-

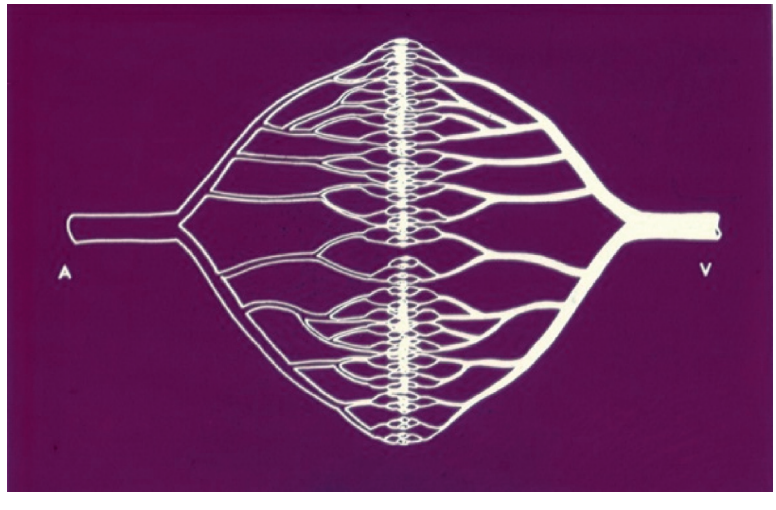

Fig 2. Esquema do sistema vascular, com macro e microcirculação. As artérias (A) ramificam-se sucessivamente até originarem capilares, após o que estes convergem na formação de veias (V).

[1] Professor catedrático aposentado e ex-director do Instituto de Bioquímica Fisiológica/Biopatologia Química da Faculdade de Medicina da Universidade de Lisboa. Sócio fundador e 1º presidente da SPHM.

dotélio e membrana basal envolvente, enquanto as vénulas de maiores dimensões (50-200 μm) incluem uma camada muscular e elástica mais fina do que a das arteríolas. A inervação simpática, extensiva somente às vénulas de maiores dimensões, influencia o respectivo tónus, com implicações na pressão hidrostática capilar, mas com escasso efeito na regulação microvascular. Um outro tipo de vénulas, cujo endotélio se caracteriza por células cubóides (donde a designação de vénulas de endotélio alto), permite, em situações de infecção, a passagem directa dos linfócitos dos capilares para os gânglios linfáticos regionais.

Os capilares, que são os vasos mais estreitos (diâmetro:5-10 μm) e pequenos da rede circulatória, estão reduzidos a uma monocamada endotelial (de epitélio escamoso), envolvida por uma camada fibrosa muito fina (membrana basal)e células contrácteis (pericitos). Não possuem adventícia nem camada média e, portanto, não têm inervação. Na transição com as arteríolas, estão localizados anéis musculares, designados como *esfíncteres pré-capilares*, dependentes do simpático.

Os capilares são o sector ideal para as trocas sangue/tecidos. Estes microvasos não actuam isolados; pelo contrário, unem-se entre si, formando uma rede embebida nos órgãos perfundidos. Esta rede é tanto mais densa quanto maior for a actividade metabólica do tecido local, de modo a assegurar as suas trocas com o sangue. As variações da pressão arterial sistémica são como que absorvidas pelas arteríolas, pelo que é mínima a sua repercussão no fluxo capilar.

A membrana basal subjacente ao epitélio capilar pode configurar três tipos, com localização electiva em determinados órgãos: contínuo, fenestrado ou descontínuo. No tipo *contínuo* (p.ex., pele, pulmões, músculo esquelético, sistema nervoso central) a membrana basal não tem interrupções de continuidade, o que limita as trocas apenas a moléculas pequenas, como as de água e iões, através das estreitas junções intercelulares. O tipo *fenestrado* (p.ex., glomérulos renais, glândulas exócrinas, mucosa intestinal) caracteriza-se por apresentar poros ou perfurações (diâmetro 60-80 nm) que possibilitam a passagem de moléculas pequenas. O tipo *descontínuo*, ou *sinusoidal* (p. ex., fígado, medula óssea, baço) é o mais permeável a moléculas de maiores dimensões (p.ex. proteínas séricas) e células sanguíneas (eritrócitos e leucócitos) através das aberturas da membrana basal (diâmetro: 30-40 μm) e dos intervalos das junções intercelulares do endotélio.

A microcirculação, além dos componentes que transportam sangue, inclui também os linfáticos e respectivos ductos colectores, a referir em separado.

Funções – A microcirculação intervém nas seguintes funções principais:

- Irrigação tecidual;
- Pressão sanguínea,
- Trocas líquidas transcapilares entre o sangue e os tecidos irrigados;
- Oxigenação tecidual, fornecimento de nutrientes e remoção de CO2 e produtos locais;
- Temperatura corporal;
- Protecção anti-inflamatória.

Estas funções estão distribuídas por três sectores funcionais distintos:

- *Sector de resistência* – representado pelas arteríolas;
- *Sector de trocas* – constituído pelos capilares (em grande parte);
- *Sector de estagnação transitória* – inclui as vénulas.

A inervação simpática dos esfíncteres pré-capilares e meta-arteríolas está na origem das contracções e relaxamentos regulares autónomos, de que resulta o fluxo intermitente capilar. Esta *vasomotilidad*e contribui, ainda, para o valor da resistência vascular periférica.

As anastomoses formadas a partir das meta-arteríolas na pele contribuem para regulação da temperatura corporal; ao aumentarem o fluxo cutâneo elevam também as perdas térmicas, ao contraírem-se, reduzem o fluxo sanguíneo local, preservando o calor corporal.

As trocas de substâncias entre o sangue e os tecidos irrigados integram, indubitavelmente, a função mais importante que, em sentido lato, fundamenta a nutrição celular. Embora a grande parte destas trocas ocorra na rede capilar, também existem trocas de líquidos e macromoléculas através das junções celulares das vénulas mais pequenas. Os electrólitos e moléculas pequenas atravessam a parede capilar através de poros, enquanto a glicose requer um sistema de transporte activo. As moléculas proteicas atravessam dificilmente os poros membranares, ocorrendo parte por pinocitose.

As moléculas de água têm a particularidade de atravessar a membrana capilar por dois mecanismos: difusão ou filtração.

A difusão transcapilar, que totaliza cerca de 80.000 L/dia (cerca de 10 vezes do que o valor do débito cardíaco e da perfusão sanguínea capilar), é bidireccional ao longo do trajecto de cada capilar. No entanto, por não existirem, normalmente, diferenças no gradiente de concentração da água entre os dois lados da membrana, o valor final do fluxo é nulo, de acordo com a *lei de Fick* (F= kA (C2-C1) /t para a difusão, em que *F*, fluxo; *k*, constante de permeabilidade da membrana a cada substância;*C2- C1*, diferença de concentração nos doía lados da membrana; *A*, área de superfície da membrana em que ocorrem as trocas);*t*, espessura da membrana), através da qual o valor da difusão de cada substância é proporcional à diferença de concentrações através da membrana e à área superficial, e inversamente proporcional à espessura dessa membrana.

As trocas transcapilares de gases respiratórios, nutrientes e produtos metabólicos, são determinadas também pela difusão, ainda que, nestes casos, o fluxo ocorra no sentido da respectiva concentração mais baixa.

Pelo contrário, a filtração transcapilar da água (com volumes muito inferiores aos da difusão) é determinada pelo desequilíbrio local entre as pressões hidrostática e oncótica (*forças de Starling*). No total, cerca de 20 L de água são movimentadas para o exterior na extremidade arterial dos capilares de todo o organismo, enquanto 18 L são reabsorvidas na extremidade venosa. Assim, a filtração num ou noutro sentido decorre ao longo do capilar em sectores distintos. A diferença residual (cerca de

2L/dia) retorna à circulação como constituinte linfático. Enquanto este processo tem particular utilidade para as trocas de água, não é quase utilizada por gases, nutrientes ou produtos metabólicos.

Entre as substâncias transferidas predominam os gases respiratórios (O2, CO_2), água, electrólitos produtos azotados, glicose, lípidos e drogas. Os gases difundem facilmente através das paredes capilares, bem como a água e lípidos. Porém, cerca de 2/3 do oxigénio difunde para os tecidos a nível das arteríolas, sobrando o restante para difusão capilar. A difusão do oxigénio para os tecidos irrigados é assegurada, em condições fisiológicas, pelo elevado gradiente a que chega aos tecidos, em média igual a 50-55 mmmHg, muito superior ao conteúdo de oxigénio intracelular (Fig.3).

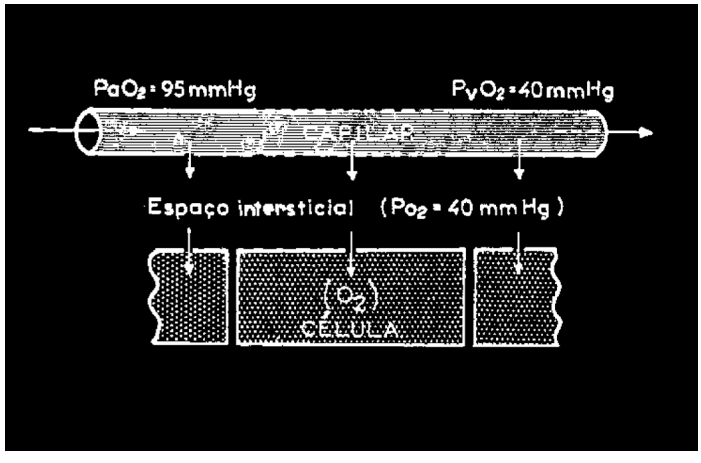

Fig.3. Diferencial da pressão de oxigénio existente, em média, no sangue arterial venoso e espaço interesticial e venoso, admitindo-se que nas células seja igual ou inferior a 5mmHg.Estas condições são extremamente favoráveis à difusão do oxigénio do sangue para os espaços celulares envolventes.

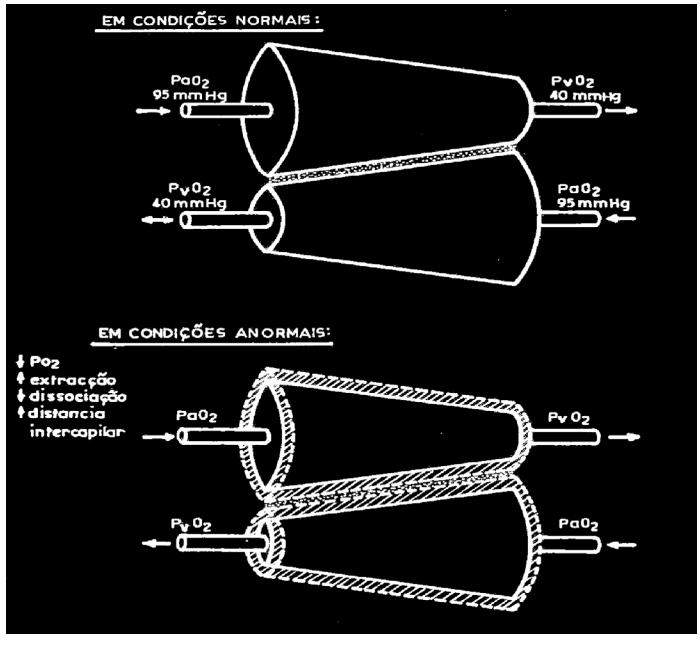

Fig.4. Modelo de oxigenação de Krogh, adaptado.

De acordo com a teoria original dos cilindros de Krogh (Fig.4), cada cone de oxigenação representaria o território teórico oxigenado por um capilar. À medida que o sangue fluisse da extremidade arterial para a venosa, diminuiria a PO_2 do sangue (de 95 para 40 mmHg), causando a redução gradual do raio de tecido oxigenado. Para compatilizar o modelo com a realidade, cada capilar como estaria em paralelo com outros, adjacentes, que transportariam o sangue em sentido inverso, entre si. Deste modo, por justaposição e orientação oposta de cones, todo o tecido seria virtualmente oxigenado pela rede capilar local, excepto a zona em que contactariam entre si, onde não haveria oxigenação ou seria muito baixa. Esta zona de hipoxia ou *anoxia* tenderia a aumentar em condições anormais, nomeadamente, por diminuição da P_2O_2, ou da extracção de oxigénio pelos tecidos, ou maior afinidade da hemoglobina para o oxigénio ou rarefacção da rede capilar local.O facto de estar estabelecido,actualmente, que a maior parte da oxigenação tecidual é iniciada a partir das arteríolas não invalida os resultados teóridos do modelo.

STENT THROMBOSIS WITH DRUG-ELUTING AND BARE-METAL STENTS: EVIDENCE FROM A COMPREHENSIVE NETWORK META-ANALYSIS.

Palmerini T, Biondi-Zoccai G, Della Riva D, Stettler C, Sangiorgi D, D'Ascenzo F, Kimura T, Briguori C, Sabatè M, Kim HS, De Waha A, Kedhi E, Smits PC, Kaiser C, Sardella G, Marullo A, Kirtane AJ, Leon MB, Stone GW[1] .

ABSTRACT

The relative safety of drug-eluting stents and bare-metal stents, especially with respect to stent thrombosis, continues to be debated. In view of the overall low frequency of stent thrombosis, large sample sizes are needed to accurately estimate treatment differences between stents. We compared the risk of thrombosis between bare-metal and drug-eluting stents. **Methods:** For this network meta-analysis, randomised controlled trials comparing different drug-eluting stents or drug-eluting with bare-metal stents currently approved in the USA were identified through Medline, Embase, Cochrane databases, and proceedings of international meetings. Information about study design, inclusion and exclusion criteria, sample characteristics, and clinical outcomes was extracted. **Findings:** 49 trials including 50,844 patients randomly assigned to treatment groups were analysed. 1-year definite stent thrombosis was significantly lower with cobalt-chromium everolimus eluting stents (CoCr-EES) than with bare-metal stents (odds ratio [OR] 0·23, 95% CI 0·13-0·41). The significant difference in stent thrombosis between CoCr-EES and bare-metal stents was evident as early as 30 days (OR 0·21, 95% CI 0·11-0·42) and was also significant between 31 days and 1 year (OR 0·27, 95% CI 0·08-0·74). CoCr-EES were also associated with significantly lower rates of 1-year definite stent thrombosis compared with paclitaxel-eluting stents (OR 0·28, 95% CI 0·16-0·48), permanent polymer-based sirolimus-eluting stents (OR 0·41, 95% CI 0·24-0·70), phosphorylcholine-based zotarolimus-eluting stents (OR 0·21, 95% CI 0·10-0·44), and Resolute zotarolimus-eluting stents (OR 0·14, 95% CI 0·03-0·47). At 2-year follow-up, CoCr-EES were still associated with significantly lower rates of definite stent thrombosis than were bare-metal (OR 0·35, 95% CI 0·17-0·69) and paclitaxel-eluting stents (OR 0·34, 95% CI 0·19-0·62). No other drug-eluting stent had lower definite thrombosis rates compared with bare-metal stents at 2-year follow-up. **Interpretation:** In randomised studies completed to date, CoCr-EES has the lowest rate of stent thrombosis within 2 years of implantation. The finding that CoCr-EES also reduced stent thrombosis compared with bare-metal stents, if confirmed in future randomised trials, represents a paradigm shift. **[Lancet. 2012 14;379(9824):1393-402].** PMID:22445239

[1] Istituto di Cardiologia, Policlinico S Orsola, Bologna, Italy.

ALTERATIONS OF THE ERYTHROCYTE MEMBRANE DURING SEPSIS

Serroukh Y, Djebara S, Lelubre C, Zouaoui Boudjeltia K, Biston P, Piagnerelli M[2]

ABSTRACT

Erythrocytes have been long considered as "dead" cells with transport of oxygen (O_2) as their only function. However, the ability of red blood cells (RBCs) to modulate the microcirculation is now recognized as an important additional function. This capacity is regulated by a key element in the rheologic process: the RBC membrane. This membrane is a complex unit with multiple interactions between the extracellular and intracellular compartments: blood stream, endothelium, and other blood cells on the one hand, and the intracytoplasmic compartment with possible rapid adaptation of erythrocyte metabolism on the other. In this paper, we review the alterations in the erythrocyte membrane observed in critically ill patients and the influence of these alterations on the microcirculatory abnormalities observed in such patients. An understanding of the mechanisms of RBC rheologic alterations in sepsis and their effects on blood flow and on oxygen transport may be important to help reduce morbidity and mortality from severe sepsis.[**Crit Care Res Pract. 2012;2012:702956. Epub 2012 May 21**] PMID:22675622

[2] Department of Intensive Care, CHU-Charleroi, Université Libre de Bruxelles, 92, Boulevard Janson, 6000 Charleroi, Belgium.

BENCH-TO-BEDSIDE REVIEW: MICROVASCULAR DYSFUNCTION IN SEPSIS-HEMODYNAMICS, OXYGEN TRANSPORT, AND NITRIC OXIDE

Bateman RM, Sharpe MD, Ellis CG[3]

ABSTRACT

The microcirculation is a complex and integrated system that supplies and distributes oxygen throughout the tissues. The red blood cell (RBC) facilitates convective oxygen transport via co-operative binding with hemoglobin. In the microcirculation oxygen diffuses from the RBC into neighboring tissues, where it is consumed by mitochondria. Evidence suggests that the RBC acts as deliverer of oxygen and 'sensor' of local oxygen gradients. Within vascular beds RBCs are distributed actively by arteriolar tone and passively by rheologic factors, including vessel geometry and RBC deformability. Microvascular oxygen transport is determined by microvascular geometry, hemodynamics, and RBC hemoglobin oxygen saturation. Sepsis causes abnormal microvascular oxygen transport as significant numbers of capillaries stop flowing and the microcirculation fails to compensate for decreased functional capillary density. The resulting maldistribution of RBC flow results in a mismatch of oxygen delivery with oxygen demand that affects both critical oxygen delivery and oxygen extraction ratio. Nitric oxide (NO) maintains microvascular homeostasis by regulating arteriolar tone, RBC deformability, leukocyte and platelet adhesion to endothelial cells, and blood volume. NO also regulates mitochondrial respiration. During sepsis, NO overproduction mediates systemic hypotension and microvascular reactivity, and is seemingly protective of microvascular blood flow. [**Crit Care. 2003 Oct;7(5):359-73. Epub 2003 Jul 28].**PMID:12974969

[3] Vascular Biology Program, Lawson Health Research Institute, The University of Western Ontario, London, Ontario, Canada. bateman@uwo.ca

NITRIC OXIDE SYNTHASE-DEPENDENT VASODILATION OF HUMAN SUBCUTANEOUS ARTERIOLES CORRELATES WITH NONINVASIVE MEASUREMENTS OF ENDOTHELIAL FUNCTION.

Dharmashankar K, Welsh A, Wang J, Kizhakekuttu TJ, Ying R, Gutterman DD, Widlansky ME[4]

ABSTRACT

Background: Noninvasive measurements of endothelial function predict future adverse cardiovascular events, but offer limited opportunities for mechanistic insights into phenotypic observations. Subcutaneous adipose arterioles, accessible through minimally invasive methods, provide an opportunity for complimentary mechanistic studies. Limited data relating subcutaneous arteriolar endothelial function, cardiovascular risk factors, and noninvasive measurements of endothelial function currently exist. **Methods:** Forty-four subjects underwent noninvasive studies of endothelial function (brachial reactivity (flow-mediated dilation (FMD) and digital pulse arterial tonometry (PAT)) and measurements of endothelial-dependent vasodilation of gluteal subcutaneous arterioles to acetylcholine. Arteriolar endothelial function was measured (i) percent vasodilation to maximal acetylcholine dose (10(-5) mol/l) and (ii) total area under the curve (AUC) for the entire acetylcholine dose-response curve (total AUC-acetylcholine (Ach), doses 10(-10)-10(-5) mol/l). **Results:**Acetylcholine responses were almost completely nitric oxide (NO) dependent. Total AUC-Ach predicted FMD and PAT, but maximal acetylcholine vasodilation was not associated with these measures. A history of hypertension, diabetes, smoking, and low-density lipoprotein cholesterol levels were independent predictors of total AUC-Ach. In regression models, total AUC-Ach independently predicted FMD.**Conclusions:** Acetylcholine vasodilator responses in human gluteal subcutaneous arterioles are NO synthase dependent and correlate with cardiac risk factors and in vivo measures of endothelial function. These data suggest subcutaneous arterioles offer an opportunity for translational studies of mechanisms of modulating NO bioavailability relevant to in vivo endothelial function measures.[Am J Hypertens. 2012;25:528-34].

[4] Department of Medicine, Division of Cardiovascular Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA.

RESUMOS DA REUNIÃO CONJUNTA

Sociedade Portuguesa de Hemorreologia e Microcirculação

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

Lisboa, 31 de Março de 2012

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

Maria do Sameiro Faria[1,2], Sandra Ribeiro[3,4], Henrique Nascimento[3,4], Petronila Rocha-Pereira[4,5], Vasco Miranda[1], Denisa Mendonça[2,7], Alexandre Quintanilha[2,4], Elísio Costa[4,6], Luís Belo[3,4], Alice Santos-Silva[3,4]

[1]FMC, Dinefro – Diálises e Nefrologia, SA – Portugal; [2]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal; [3]Faculdade Farmácia, Serviço de Bioquímica, Universidade do Porto – Portugal; [4]Instituto Biologia Molecular e Celular (IBMC), Universidade do Porto – Portugal; [5]Centro Investigação Ciências Saúde (CICS), Universidade Beira Interior, Covilhã – Portugal; [6]Instituto de Ciências da Saúde da Universidade Católica Portuguesa, Porto – Portugal; [7]Instituto de Saúde Pública da Universidade do Porto (ISPUP), Porto, Portugal

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

Filipa Mascarenhas Melo[1], Filipe Palavra[2], Edite Teixeira de Lemos[1,3], José Sereno[1], Daniela Marado[4,5], Frederico Teixeira[1], Flávio Reis[1]

[1]Laboratório de Farmacologia e Terapêutica Experimental, IBILI, Faculdade de Medicina de Coimbra; [2]Serviço de Neurologia do Centro Hospitalar de Coimbra (E.P.E.); [3]Centro de Estudos em Educação, Tecnologias e Saúde, ESAV, Instituto Politécnico de Viseu; [4]Serviço de Medicina e [5]Hospital de Dia de Diabetes e Doenças Metabólicas do Centro Hospitalar de Coimbra (E.P.E.).

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.

Agradecimentos: FCT-COMPETE (SFRH/BD/65483/2009).

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

Sérgio Teixeira, Joana Martins, Pedro Sá Pinto, Clara Nogueira, Carolina Vaz, Tiago Loureiro, Luís Loureiro, Diogo Silveira, Duarte Rego, Rui de Almeida.

Serviço de Angiologia e Cirurgia Vascular, Centro Hospitalar do Porto – Hospital Geral de Santo António

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

Susana Coimbra 1,2, Jorge Brandão Proença 3, Alice Santos-Silva 1,4, Maria João Neuparth 2,5

1Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal; 2Centro de Investigação das Tecnologias da Saúde (CITS) – Instituto Politécnico da Saúde Norte (IPSN), Gandra-Paredes, Portugal; 3Instituto Superior de Ciências da Saúde Norte (ISCSN), Gandra-Paredes, Portugal; 4Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 5Centro de Investigação em Atividade Física, Saúde e Lazer (CIAFEL), Universidade do Porto, Porto, Portugal.

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"

Patrícia Napoleão[1,2], Mafalda Selas[3], Cláudia Freixo[3], Catarina Ramos[1], Miguel Mota Carmo[2,3], Ana Maria Viegas-Crespo[4], Rui Cruz Ferreira[3], Teresa Pinheiro[1,5]

[1] Grupo de Estudos Biomédicos, Unidade de Física e Aceleradores, Instituto Tecnológico e Nuclear, Sacavém [2] Centro de Estudos de Doenças Crónicas, Faculdade Ciências Médias, Universidade Nova de Lisboa, Lisboa, Portugal [3] Serviço Cardiologia, Hospital Santa Marta, Lisboa, Portugal [4] CESAM & Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal [5] Centro de Física Nuclear, Universidade Lisboa, Portugal.

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"

José Sereno[1], Ana M. Romão[1], Belmiro Parada[1,2], Cristina Mega[1,3,4], Helena Vala[3,4], Edite Teixeira de Lemos[1,3], Frederico Teixeira[1], Flávio Reis[1]

[1]Laboratory of Pharmacology & Experimental Therapeutics, IBILI, Medicine Faculty of Coimbra; [2]Dept. of Urology & Renal Transplantation, CHUC; [3]ESAV and [4]Educational, Technologies and Health Study Center, Polytechnic Institute of Viseu.

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).

Acknowledgements: Lab. Pfizer Lda and FCT-Compete (SFRH/BD/63962/2009).

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

Henrique Nascimento[1,2], Susana Casal[3], Luísa Aires[4], Susana Rocha[1,2], João Fernandes[1,2], Elísio Costa[2,5], Carla Rego[6], Helena Ferreira Mansilha[7], Petronila Rocha-Pereira[8], Alexandre Quintanilha[2,9], Luís Belo[1,2], Alice Santos-Silva[1,2].

[1]Departamento de Bioquímica, Faculdade de Farmácia, Univ. Porto; [2]Instituto de Biologia Molecular e Celular (IBMC), Univ. Porto; [3]REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Univ. Porto; [4]Centro de Investigação em Actividade Física Saúde e Lazer (CIAFEL) – Faculdade de Desporto, Univ.

Porto; [5]Escola Superior de Biotecnologia, Universidade Católica Portuguesa; [6]Unidade de Nutrição / Serviço Pediátrico. UAG-MC. Hospital de S. João E.P.E. Faculdade de Medicina, Univ. Porto; [7]Departamento Pediátrico, Centro Hospitalar do Porto – Hospital de Crianças D. Maria Pia; [8]Centro de Investigação em Ciências da Saúde, Univ. Beira Interior; [9]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Univ. Porto.

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

Saldanha C, Freitas T, Lopes de Almeida JP
Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa
carlotasaldanha@fm.ul.pt

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

Carlota Saldanha
Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa
carlotasaldanha@fm.ul.pt

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.
Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, Portugal. vandaalmeida@fm.ul.pt

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.

de Almeida JP, Oliveira S, Saldanha C.
University of Lisbon Medical School, Santa Maria Hospital, Lisbon, Portugal Institute of Biochemistry, Institute of Molecular Medicine, University of Lisbon Medical School, Lisbon, Portugal.

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.

de Oliveira P, Gomes AQ, Pacheco TR, Vitorino de Almeida V, Saldanha C, Calado A.
Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal.

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.

Lopes de Almeida JP, Freitas-Santos T, Saldanha C.
University of Lisbon Medical School, Santa Maria Hospital, Lisbon, Portugal. jpedro.gla@gmail.com

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.

Saldanha C, de Almeida JP.

Source

University of Lisbon Medical School, Santa Maria Hospital, Lisbon, Portugal. carlotasaldanha@fm.ul.pt

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.

de Almeida JP, Freitas-Santos T, Saldanha C.

University of Lisbon Medical School, Santa Maria Hospital, Lisbon, Portugal. jpedro.gla@gmail.com

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.

De Oliveira S, Vitorino de Almeida V, Calado A, Rosário HS, Saldanha C.
Universidade de Lisboa, Lisboa, Portugal.

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.

Saldanha C, Loureiro J, Moreira C, Silva JM.

Instituto de Medicina Molecular, Unidade de Biologia Microvascular e Inflamação, Instituto de Bioquímica Faculdade de Medicina da Universidade de Lisboa. Av Prof. Egas Moniz, 1649-028 Lisboa, Portugal.

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.

Santos MJ, Pedro LM, Canhão H, Fernandes e Fernandes J, Canas da Silva J, Fonseca JE, Saldanha C.

Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisbon, Portugal. mjps@netvisao.pt

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].**

REGRAS DE PUBLICAÇÃO / INSTRUCTIONS TO AUTHORS

CONVITE

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INSTRUÇÕES

1. Todos os textos enviados para publicação estão sujeitos a apreciação editorial e aprovação. A decisão é baseada no mérito científico e cultural dos trabalhos.

2. São aceites somente os trabalhos preparados em versão óptica (*PDF* ou *Microsoft Word*).

3. Os textos devem ser redigidos em Português ou Inglês.

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