BOLETIM

Sociedade Portuguesa de Hemorreologia e Microcirculação

Bulletin of the Portuguese Society of Hemorheology and Microcirculation

Vol. 24 n.º 3 Julho, Agosto, Setembro 2009

Sumário / Summary

Sociedade Portuguesa de Hemorreologia e Microcirculação

Presidente Honorário: Prof. Doutor João Alcindo Martins e Silva

ÓRGÃOS SOCIAIS DA SPHM / BOARDS (2007-2009)

Direcção / Executive Committee

Presidente
Prof.ª Doutora Maria Carlota Saldanha Lopes

Vice-Presidentes
Prof. Doutor J. M. Braz Nogueira
Prof. Doutor Carlos Perdigão

Secretário-Geral
Dr. José António Pereira Albino

Tesoureiro
Prof. Doutor Flávio Reis

Secretários-Adjuntos
Prof. Doutor Henrique Luz Rodrigues
Prof. Doutor J. Ducla Soares
Dr. Jorge Lima

Assembleia Geral / General Assembly

Presidente
Prof. Doutor A. Diniz da Gama

1.º Secretário
Dr. João Paulo Guimarães

2.º Secretário
Dr. Miguel Frederico Leal Galvão

1.º Secretário Suplente
Dr. Luís Sargento

2.º Secretário Suplente
Dr. Paulo Ferreira da Silva

Conselho Fiscal / Finance and Audit Committee

Presidente
Prof. Doutor Victor Oliveira

1.º Vogal
Dr.ª Maria Helena Baptista Manso Ribeiro

2.º Vogal
Dr. Carlos Manuel dos Santos Moreira

Comissão de Delegados / Committee of Delegates

Delegado da Região Norte
– Dr. Manuel Campos
Delegado da Região Centro
– Dr. João Morais
Delegado da Região Sul e Regiões Autónomas
– Dr. Mário Marques

MEMBROS CONSULTIVOS, HONORÁRIOS E CORRESPONDENTES / / CONSULTIVE, HONORARY AND CORRESPONDENT MEMBERSHIP

Conselho Científico / / Scientific Council

A. Diniz da Gama
Axel Pries
Fernando Lacerda Nobre
Helena Saldanha Oliveira
J. Esperança Pina
J. Luís Providência
J. Martins e Silva
J. Fernandes e Fernandes
J. Rafael Ferreira
João Morais
José Ferro
Luís Silva Carvalho
Manuel Carrageta
Mário Andreia
Ricardo Seabra Gomes

Sócios Honorários / / Honorary Members

A. M. Ehrly (Alemanha)
Carlos Ribeiro (Portugal)
H. J. Meiselman (EUA)
Helmut Drexler (Alemanha)
J. F. Stoltz (França)
J. E. Tooke (G. Bretanha)
John A. Dormandy (G. Bretanha)
Joaquim Silva Carvalho (Portugal)
J. M. G. Toscano Rico (Portugal)
L. Teixeira Diniz (Portugal)
M. Boisseau (França)
Políbio Serra e Silva (Portugal)
Sandro Forconi (Itália)
Y. Isogai (Japão)

Sócios Correspondentes / / Correspondent Member

Adrian J. Barnes (G. Bretanha)
Alon Harris (USA)
D. Seiffge (Alemanha)
G. Caimi (Itália)
G. D. O. Lowe (G. Bretanha)
I. Juhan-Vague (França)
I. Salama Benarroch (Argentina)
J. Delaunay (França)
J. F. Brun (França)
Ricardo Manrique (Brasil)
Shi Yong-de (China)
T. Shiga (Japão)
Tao Chan (França)

FILIAÇÃO INTERNACIONAL

EUROPEAN SOCIETY FOR CLINICAL HEMORHEOLOGY
EUROPEAN SOCIETY FOR MICROCIRCULATION

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)

Esta publicação é subsidiada em 2009 por:
FCT: Fundação para a Ciência e Tecnologia (Ministério da Ciência e do Ensino Superior – Portugal)
Ao abrigo do: **Apoio do Programa Operacional Ciência, Tecnologia, Inovação do Quadro Comunitário de Apoio III**

O Boletim (ISSN 0872-4938) é publicado trimestralmente pela Sociedade Portuguesa de Hemorreologia e Microcirculação. Isenta de registo no ICS nos termos da alínea a) do n.º 1 do artigo 12.º do Decreto Regulamentar n.º 8/99, de 9 de Junho. **Depósito Legal** 30 525/89. **Tiragem** 100 exemplares **Distribuição** sócios, sociedades científicas afins, entidades oficiais e privadas de âmbito médico e áreas de educação da ciência. Todos os direitos estão reservados. **Preço de cada número avulso:** 5 €, a que acresce 2,5 € para portes de correio. **Editor, Proprietário, Administração e Secretariado:** Sociedade Portuguesa de Hemorreologia e Microcirculação, a/c Instituto de Biopatologia Química, Faculdade de Medicina de Lisboa. **Endereço do Secretariado:** Apartado 4098- 1502 Lisboa Codex, Portugal. **Telefone** 217 985 136; Fax: 219 999 477 **Execução Gráfica:** Publicações Ciência e Vida, Lda. Apartado 44 – 2676-901 Odivelas. **Telef.:** 21 478 78 50; **Fax:** 21 478 78 59. **E-mail:** pub@cienciaevida.pt

CONSELHO DE AVALIADORES

A nossa sociedade aproveitou a realização, em 2004 da "*23rd European Conference on Microcirculation* "para criar um *website* para divulgação e acompanhamento do evento. Este sítio (*www.hemorreologia.com*) contém toda a documentação sobre a SPHM, nomeadamente o que é, quais os seus objectivos, a composição dos órgãos de gestão, actualizações temáticas, noticiário, *"links"* e o *Boletim* da SPHM. A inclusão deste diminuiu as despesas de impressão, as quais actualmente ainda ficam aquém do montante recebido da Fundação para a Ciência e Tecnologia (FCT) e quotização por parte dos sócios. A feitura de alguns exemplares mantém-se para distribuição pelas entidades oficiais e bibliotecas.

A continuação do apoio financeiro por parte da FCT exige que o Boletim possua um Conselho de Avaliadores (aqui entendido como *Conselho Editorial Internacional)*e que o modo de funcionamento do mesmo esteja divulgado no Boletim. Esta mudança, imposta, vai ao encontro da vontade dos órgãos de gestão da SPHM. Naturalmente, valorizará cientificamente a publicação oficial da SPHM e ainda atrairá leitores e autores. O número de visitantes do nosso site não para de aumentar e, só para termos uma ideia, dando o número registado em 21 de Setembro de 2009 (312 163 visitantes) será decerto ultrapassado aquando da vossa leitura deste número do *Boletim*.

O Conselho de Avaliadores integra cientistas oriundos de vários cantos do globo tal como, Bulgária, Estados Unidos da América, França, Japão, Portugal e Turquia. Os CV e fotos dos novos membros serão divulgados no próximo números. De imediato apraz-me, em nome da SPHM, agradecer a todos a aceitação da tarefa de avaliar os manuscritos de natureza diversa, designadamente, artigos originais, revisões a cartas ao editor. A participação do Conselho de Avaliadores em *peer review* prestigia o Boletim, sendo novo motivo de agradecimento pelo tempo que dispensarão na apreciação dos manuscritos, porque acontecerá terem de interromper o trabalho ou tempo de lazer para entrarem no domínio do tema. A actualização e pertinência do que se publica assim o exigem.

Ao introduzirmos este mecanismo de controlo de qualidade estamos a validar o significado e a originalidade do conhecimento produzido e ou sistematizado ou integrado. O espectro de leitores, no que respeita à formação e hábitos de leitura, é seguramente amplo e a mudança agora anunciada expressa a nossa vontade de continuar a oferecer oportunidades de difusão do saber qualificado.

O Conselho de Avaliadores gera um processo de acompanhamento e revisão da produção científica dos autores que, na actualidade, responde com rapidez e facilidade de correcção, apoiado pelos meios electrónicos que pretendemos colocar em acção.

Estamos certos que os autores continuarão a apostar no envio dos seus manuscritos, que serão revistos e comentados, quando necessário, para os melhorar. Temos que estar todos agradecidos a esta mudança e aos seus intervenientes

Desejo que o Conselho de Avaliadores seja um estímulo ao envio de manuscritos.

Carlota Saldanha
Presidente da SPHM

POLYMORPHONUCLEAR LEUKOCYTE RHEOLOGY, CYTOSOLIC CA^{2+} CONTENT, BETA$_2$-INTEGRIN EXPRESSION AND OXIDATIVE STRESS IN HYPERTENSION

Gregorio Caimi, Eugenia Hopps, Rosalia Lo Presti

INTRODUCTION

An elevated leukocyte count is associated to arterial hypertension, but in this brief review we focus our attention on some functional aspects of polymorphonuclear leukocytes (PMNs), considering in particular our data previously published on this topic. Leukocytes have a likely role in the pathogenesis of organ injury accompanying arterial hypertension. Monocytes and neutrophils can in fact participate in the progression of vascular lesions and in the pathophysiology of their ischaemic complications.

LEUKOCYTE RHEOLOGY

Hypertensives have a whole-blood hyperviscosity, due to increased leukocyte count, erythrocyte rigidity and hematocrit, and also to a raise of plasma viscosity, fibrinogen concentration, erythrocyte and platelet aggregation. As blood viscosity influences peripheral vascular resistances, changes of the haemorheological parameters may contribute to the development of hypertension and can facilitate myocardial and vascular remodelling[1].

In hypertensives the reduced membrane fluidity of blood cells affects carrier activity: in PMNs alterations of sodium and calcium plasma membrane pumps have been observed. Higher sodium cytosolic concentrations promote leukocyte activation, superoxide anion synthesis, adhesion to endothelium and progressive organ injury[2]. Several researches have previously demonstrated normal cytosolic Ca^{2+} concentrations in hypertensive monocytes and PMNs[3-5]. On the contrary, we observed increased cytosolic Ca^{2+} concentrations in PMNs[6], as well as in platelets and erythrocytes[7]. This datum was confirmed by more recent studies in insulin-resistant hypertensive men[8].

Dipartimento di Medicina Interna, Malattie Cardiovascolari e Nefrourologiche
Cattedra di Semeiotica e Metodologia Medica
Università di Palermo, Italy

Corresponding author:
Prof. Gregorio Caimi
Via Leonardo da Vinci, 52
90145 Palermo, Italy
Fax: +39 91 6554535; *e-mail:* caimigre@unipa.it

In our research, at baseline, no correlation was present between PMN membrane fluidity and cytosolic Ca^{2+} content or between membrane fluidity and arterial pressure in hypertensives[7,9], whereas PMN membrane fluidity was decreased in hypertensives compared to normals[10]. After *in vitro* activation with PMA the initial relative flow rate (obtained by filtering unfractionated WBC and PMN suspension using a St. George's Filtrometer) was decreased in normals and hypertensives, but no other modifications were found. After activation with fMLP, Ca^{2+} content was furtherly increased only in hypertensives[11,12].

LEUKOCYTE ACTIVATION

In hypertensives, leukocytes have higher activation levels than in normal subjects[13]. Stimulated granulocytes release reactive oxygen species (ROS) which may inactivate nitric oxide (NO), arachidonic acid metabolites which induce vascular inflammation, and cathepsin G which stimulates the angiotensin system[14]. Primed PMNs may also initiate platelet aggregation[14] and provoke leukocyte adhesion to the endothelium promoting the hyperexpression of adhesion molecules[15], and the NO inactivation by superoxide anion. NO is in fact an endogenous inhibitor of leukocytes adhesion in postcapillary venules[16].

Subjects with essential hypertension have increased plasma levels of leukocyte elastase[17,18], that is considered a sensitive diagnostic marker of cardiovascular disease. Higher elastase levels are associated with the presence of atheromatous plaques[19] and are related to arterial stiffness[18]. In spontaneously hypertensive rats (SHRs) circulating PMNs have lower alkaline phosphatase content, while the plasma levels of this enzyme are elevated in these models[16,20]; this finding is another indicator of leukocyte activation and spontaneous degranulation and reflects the alteration of leukocyte function too.

LEUKOCYTES AND OXIDATIVE STRESS

Leukocytes activated by soluble or cellular stimuli start the respiratory burst, characterized by increased oxygen consumption and generation of superoxide (O_2^-), hydrogen peroxyde (H_2O_2) and other ROS. O_2^- synthesis by hypertensive PMNs is related to blood pressure [21]. ROS contribute to hypertension and its complications causing endothelial damage, oxidizing circulating low density lipoproteins and degrading NO. Serum NO levels and arterial pressure are negatively correlated[22]. The decreased availability of NO has negative effects on vascular tone and influences platelet aggregation, leukocyte adhesion and expression of adhesion molecules[23]. Oxidative stress in PMNs is related to arterial mean pressure, as well as oxidative stress in monocytes is related to C-reactive protein (CRP)[24].

A research regarding Sabra hypertension-resistant rats (SBN) and hypertension-prone rats (SBH) has shown that in pre-hypertensive SBH oxidative stress and primed PMNs are present. After salt-loading for four weeks, SBH rats developed hypertension and their PMNs pro-

duce elevated levels of ROS[25]. A long-term antioxidant treatment with Tempol (a membrane-permeable SOD mimetic), initiated in pre-hypertensive SHR, can attenuate vascular oxidative stress and prevent the age-related elevation of blood pressure in this animal model of genetic hypertension[26].

A recent study revealed a spontaneous up-regulation of CD11a and CD18 in circulating PMNs and an increased ROS generation by T-lymphocytes and monocytes in the peripheral blood of obese Zucker rats, before the development of hypertension[27]. This data suggest that primed PMNs, oxidative stress and inflammation anticipate and possibly contribute to the development of hypertension and its complications.

In Sabra rats salt-loaded for sixty days, the increase of blood pressure can be inhibited by phenylarsine oxide, an NADPH oxidase inhibitor. This finding suggests that primed PMNs play a role in the development of hypertension via activation of NADPH oxidase and production of ROS[28]. Because the leukocyte NADPH oxidase is localized in the plasma membrane, the alterations of its activity may be related to the membrane abnormalities observed in hypertension[29].

LEUKOCYTE INTEGRINS

In hypertensives, the increased expression of adhesion molecules on endothelial cells and leukocytes promotes leukocyte adhesion to the endothelium[30]. A research on SHR has demonstrated a decreased endothelial expression of P-selectin[31]. On the contrary, another study showed a venular P-selectin hyperexpression that is considered responsible for an increased rolling of PMNs[32]. The increased level of soluble E- and P-selectin in malignant and renovascular hypertension is due to platelet activation[33]. E-selectin, an indicator of organ injury, is related to blood pressure even in hypertensives without complications[34]. Benidipine, a calcium channel blocker, decreases arterial pressure and the concentrations of E- and P-selectin[35]. In coronary endothelial cells derived from human heart, angiotensin II induces a concentration-dependent increase in E-selectin expression via activation of AT1 receptors. So, the E-selectin-mediated leukocyte adhesion to the endothelium can be blocked using AT1 receptor antagonists[36]. The infusion of angiotensin II *in vivo* induces high levels of soluble ICAM-1 (Intracellular Adhesion Molecules-1) in hypertensives and normals[37]. Hypertension increases cytokine expression and concentrations of ICAM-1 and MCP-1 (monocyte chemoattractant peptide-1), responsible for the inflammatory cell infiltration of the subendothelium[38]: exposing mouse carotid arteries at high intraluminal pressure, after 24 hours an increased mRNA expression of MCP-1, IL-6, chemokines and VCAM-1 (Vascular Cell Adhesion Molecule-1) is detectable in monocytes[39]. VCAM-1 is positively related to systolic blood pressure[40]. The induction of hypertension in genetically normal rats can lead to an overexpression of the CD18 integrin on circulating helper T-cells[41]. In obese hypertensives an increased expression of CD11b on circulating monocytes and of CD68 on macrophages

in the adipose tissue was found[42]. Studies evaluating PMN beta$_2$--integrins showed at baseline a phenotypical hyperexpression of CD11a, CD11b and CD18, but not of CD11c. After *in vitro* activation an increase of CD11b, CD11c and CD18 has been demonstrated, but also a decrease of CD11a, especially with PMA[43]. The integrin overexpression on PMNs at baseline can be due to the spontaneous granulocyte activation. The fall in CD11a expression after activation might be due to different mechanisms: because CD11a is constitutively expressed on PMN surface and it is not internalized during activation, its decrease may be related to an altered cleavage or to a dysregulated phosphorylation/dephosphorylation process[44]. However, up to now the mechanisms that explain the integrin pattem are only partially known.

REFERENCES

1. Shliakhto EV, Moiseeva OM, Liasnikova EA, Villeval'de SV, Emel'ianov IV. Rheological properties of blood and endothelial function in patients with hypertensive disease. Kardiologiia 2004; 44:20-23.
2. Schmid-Schonbein GW, Seiffge D, DeLano FA, Shen K, Zweifach BW. Leukocyte counts and activation in Spontaneously Hypertensive and Normotensive Rats. Hypertension 1991;17:323-330.
3. Lew PD, Favre L, Waldvogel FA, Vallotton MB. Cytosolic free calcium and intracellular calcium stores in neutrophils from hypertensive subjects. Clin Sci 1985; 69:227-230.
4. Shore AC, Beynon GW, Jones JC, Markandu ND, Sagnella GA, MacGregor GA. Mononuclear leukocyte intracelluar free calcium – Does it correlate with blood pressure? J Hypertens 1985; 3:183-187.
5. Bing RF, Heagerty AM, Jackson JA, Thurston H, Swales JD. Leukocyte ionized calcium and sodium content and blood pressure in humans. Hypertension 1986; 8:483-488.
6. Caimi G, Canino B, Montana M, Ventimiglia G, Catania A, Lo Presti R. Polymorphonuclear leukocyte membrane fluidity and cytosolic Ca2+ content in different clinical conditions. Clin Hemorheol Microcirc 1997; 17:217-223.
7. Caimi G, Contomo A, Serra A, Catania A, Lo Presti R, Sarno A, Cerasola G. Red cell metabolic parameters and rheological determinants in essential hypertension. Clin Hemorheol 1993; 13:35--44.
8. Sela S, Shurtz-Swirski R, Farah R, Levy R, Shapiro G, Chezar J, Shasha SM, Kristal B. A link between polymorphonuclear leukocyte intracellular calcium, plasma insulin and essential hypertension. Am J Hypertens 2002; 15:291-295.
9. Caimi G. Erythrocyte, platelet and polymorphonucelar leukocyte membrane dynamic properties in essential hypertension. Clin Hemorheol Microcirc 1997; 17:199-208.
10. Lo Presti R, Carollo C, Canino B, Montana M, Romano A, Catania A, Caimi G. Poly--morphonuclear leukocyte membrane fluidity and cytosolic Ca2+ content at baseline and after activation in essential hypertension. Trace Elem Electrolytes 2005; 22:207-210.
11. Caimi G, Lo Presti R, Canino B, Montana M, Ferrara L, Oddo G, Vetimiglia G, Cerasola G. Essential hypertension: leukocyte rheology and polymorphonuclear cytosolic Ca2+ content at baseline and after activation. Clin Hemorheol Microcirc 1998; 19:281-289.
12. Caimi G, Lo Presti R, Canino B, Montana M, Cerasola G. Essential hypertension: polymorphonuclear leukocyte membrane fluidity at baseline and after chemotactic activation. Am J Hypertens 1999; 12:947-948.
13. Chatterjee M, Saluja R, Kanneganti S, Chinta S, Dikshit M. Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. Cell Mol Biol 2007; 15:84-93.
14. Mugge A, Lopez JAG. Do leukocytes have a role in hypertension? Hypertension 1991; 17:331--333.
15. Pardon NJ, Wilkinson R, Thomas TH. Rapid fusion of granules with neutrophil cell membranes in hypertensive patients may increase vascular damage. Am J Hypertens 2001; 14:927-933.
16. Shen K, Sung KL, Whittemore, DeLano FA, Zweifach BW, Schmid-Schonbein GW. Properties of circulating leukocytes in spontaneously hypertensive rats. Biochem Cell Biol 1995; 73:491--500.
17. Jackson MH, Collier A, Nicoll JJ, Muir AL, Dawes J, Clarke BF, Bell D. Neutrophil count and activation in vascular disease. Scott Med J 1992; 37:41-43.
18. Yasmin SW, McEniery CM, Dakham Z, Pusalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR, Wilkinson IB. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler Thromb Vasc Biol 2005; 25:372--378.
19. Amaro A, Gude F, Gonzalez-Juanatery R, Iglesias C, Femandez-Vasquez F, Garcia-Acuna J, Gil M. Plasma leukocyte elastase concentration in angiographically diagnosed coronary artery disease. Eur Heart J 1995; 16:615-622.
20. Kristal B, Shurtz-Swirski R, Chezar J, Manaster J, Levy R, Shapiro G, Weissman I, Shasha SM, Sela S. Participation of peripheral polymorphonuclear leukocytes in the oxidative stress and inflammation in patients with essential hypertension. Am J Hypertens 1998; 11:921-928.
21. Mazor R, Shurtz-Swirski R, Sela S, Shapiro G, Kristal B. Polymorphonuclear leukocyte priming

and counts are in correlation with blood pressure parameters. Harefuah 2006; 145:900-903.
22. Armas-Padilla MC, Armas-Hernández MJ, Sosa--Canache B, Cammarata R, Pacheco B, Guerrero J, Carvajal AR, Hernández-Hernández R, Israili ZH, Valasco M. Nitric oxide and malondialdehyde in human hypertension. Am J Ther 2007; 14:172-176.
23. Portaluppi F, Boari B, Manfredini R. Oxidative stress in essential hypertension. Curr Pharm Des 2004; 10:1695-1698.
24. Yasunari K, Maeda K, Nakamura M, Yoshikawa J. Oxidative stress in leukocyte is a possible link between blood pressure, blood glucose and C-reactive protein. Hypertension 2002; 39:777-780.
25. Sela S, Mazor R, Amsalam M, Yagil C, Yagil Y, Kristal B. Primed polymorphonuclear leukocytes, oxidative stress, and inflammation antecede hypertension in the Sabra rat. Hypertension 2004; 44:764-769.
26. Nabha L, Garbern JC, Buller CL, Charpie JR. Vascular oxidative stress precedes high blood pressure in spontaneously hypertensive rats. Clinical and Experimental Hypertension 2005; 27:71-82.
27. Kim CH, Vaziri ND, Rodriguez-Iturbe B. Integrin expression and H2O2 production in circulating and splenic leukocytes of obese rats. Obesity 2007; 15:2209-2216.
28. Mazor R, Kristal B, Cohen-Mazor M, Yagil C, Yagil Y, Sela S. The polymorphonuclear leukocyte contributes to the development of hypertension in the Sabra rat. J Hypertens 2007; 25: 2249-2256.
29. Sagar S, Kallo IJ, Kaul N, Ganguly NK, Sharma BK. Oxygen free radicals in essential hypertension. Mol Cell Biochem 1992; 111:103-108.
30. Buemi M, Allegra A, Aloisi C, Corica F, Alonci A, Ruello A, Montalto G, Frisina N. Cold pressor test raises serum concentrations of ICAM-1, VCAM-1 and E-selectin in normotensive and hypertensive patients. Hypertension 1997; 30:845--847.
31. Suzuki H, Zweifach BJ, Forrest MJ, Schmid--Schonbein GW. Modification of leukocyte adhesion in spontaneously hypertensive rats by adrenal corticosteroids. J Leuk Biol 1995; 57: 20-27.
32. Suematsu M, Suzuki H, Tamatani T, Iigou Y, DeLano FA, Miysaka M, Forrest MJ, Kannagi R, Zweifach BJ, Ishimura Y, Schmid-Schonbein GW. Impairment of Selectin-mediated leukocyte adhesion to venular endothelium in spontaneously hypertensive rats. J Clin Invest 1995; 96:2009--2016.
33. Verhaar MC, Beutier JJ, Gaillard CA, Koomans HA, Fijnheer R, Rabelink TJ. Progressive vascular damage in hypertension is associated with increased levels of circulating P-Selectin. J Hypertens 1998; 16:45-50.
34. Miller MA, Kerry SM, Cook DG, Cappuccio FP. Cellular adhesion molecules and blood pressure: interaction with sex in a multi-etnic population. J Hypertens 2004; 22:705-711.
35. Sanada H, Midorikawa S, Yatabe J, Sasaki Yatabe M, Katoh T, Baba T, Hashimoto S, Watanabe T. Elevation of serum soluble E- P-selectin in patients with hypertension is reversed by benidipine, a long-acting calcium channel blocker. Hypertens Res 2005; 28:871-878.
36. Grafe M, Auch-Schwelk W, Zakrewicz A, Regitz--Zagrosek V, Bartsch P, Graf K, Loebe M, Gaethgens P, Fleck E. Angiotensin II-induced leukocyte adhesion on human coronary endothelial cells is mediated by E-selectin. Circ Res 1997; 81:801-811.
37. Pastore L, Tessitore A, Martinotti S, Toniato E, Alesse E, Bravi MC, Ferri G, Desideri A, Gulino A, Santucci A. Angiotensin II stimulates intercellular adhesion molecule-1 (ICAM-1) expression by human vascular endothelial cells and increases soluble ICAM-1 release in vivo. Circulation 1990; 100:1646-1652.
38. Madej A, Okopien B, Kowalski J, Haberka M, Herman ZS. Plasma concentrations of adhesion molecules and chemokines in patients with essential hypertension. Pharmacol Rep 2005; 57:878-881.
39. Riou S, Mees B, Esposito B, Merval R, Vilar J, Stengel D, Ninio E, van Haperen R, de Crom R, Tedgui A, Lehoux S. High pressure promotes monocyte adhesion to the vascular wall. Circ Res 2007; 100:1226-1233.
40. De Souza CA, Dengel DR, Macko RF, Cox K, Seals DR. Elevated levels of circulating cell adhesion molecules in uncomplicated essential hypertension. Am J Hypertens 1997; 10:1335-1341.
41. Kim CH, Vaziri ND. Hypertension promotes integrin expression and reactive oxygen species generation by circulating leukocyte. Kidney Int 2005; 67:1462-1470.
42. Boschmann M, Engeli S, Adams F, Gorzelniak K, Franke G, Klaua S, Kreuzberg U, Luedtke S, Kettritz R, Sharma AM, Luft FC, Jordan J. Adipose tissue metabolism and CD11b expression on monocytes in obese hypertensives. Hypertension 2005; 46:130-136.
43. Caimi G, Lo Presti R, Carollo C, Musso M, Porretto F, Canino B, Catania A, Cerasola G. Polymorphonuclear integrins, membrane fluidity and cytosolic Ca2+ content after activation in essential hypertension. Hypertension 2000; 36:813-817.
44. Caimi G. Polymorphonuclear leukocyte integrin profile in essential hypertension. Am J Hy-pertens 2000; 13:1051-1052.

HEMORHEOLOGY AND MICROCIRCULATION IN HEALTHY AND DISEASED LIVER AND AFTER LIVER TRANSPLANTATION: A REVIEW

G. Cicco, R. Memeo, L. Lupo, S. Cicco, V. Memeo.

ABSTRACT

A good knowledge of the liver microcirculation, spanning the arterioles, the venules and the hepatic sinusoids that maintain perfusion in continuous homeostasis in healthy liver, is essential. This vascular system also includes the portal vein and it is on this blood that the liver works as a filter, protecting the subject from toxic substances derived from food, drugs, and fluids present in blood. Another liver function is to increase the general circulation output within 3-4 minutes following an emergency (shock). About 350 ml of blood are present in special hepatic storage areas.

During liver diseases (e.g. cirrhosis) there is a strong decrease in the blood hemoglobin concentration, with anemia and impairment of the liver perfusion causing a high level of tissue hypoxia. Alterations in erythrocytes and leukocytes deformability and aggregability are also present, that contribute to the impairment of the liver microcirculation.

In liver transplantation the main risk is the reperfusion injury syndrome. In fact, a sudden gush of oxygenated blood with large amounts of xanthine, in the presence of xanthine oxidase, can stimulate reactive oxygen species (ROS) production.

More studies are needed to evaluate the liver hemorheology and microcirculation and improve the diagnosis and treatment of liver disease.

Key-words: Microcirculation, hemorheology, cirrhosis, liver transplantation, ischemic reperfusion injury.

LIVER MICROCIRCULATION

Introduction

The liver microcirculation includes the terminal portal venules and the terminal hepatic arterioles (afferent vessels), the sinusoids (capillaries) and the terminal hepatic venules (efferent vessels) [1-2]. Portal blood accounts for 70-75% of the blood flow through the liver, while 25% derives from the hepatic arterioles and is modulated by the liver metabolism. A decreased oxygenation of the liver will increase the arterial blood flow

Hemorheology and Microcirculation Research Unit
D.E.T.O. – Gen. Surgery and Liver Transplantation
University of Bari – Bari – Italy.

because the need for greater liver oxygenation induces an important local vasodilator effect[3-4]. The sinusoidal blood flow presents as a series of rays stemming from the periportal area (zone 1) and the intermediate zone (zone 2) and reaching the pericentral area (zone 3), where it drains into the terminal hepatic venules[1]. This circuit, named the "liver acinus", is formed by the portal vessel in the center and the terminal hepatic venules at the periphery[2-5].

Blood Flow through the liver

About 1100 ml of portal blood enters the liver per minute. This blood flows through the hepatic sinusoids, closely linked with the hepatic parenchymal cells, and into the central liver veins, and then finally into the inferior vena cava. In addition to the portal blood, a further 350 ml of blood per minute reaches the liver from the hepatic artery, so the total liver blood flow is nearly 1500 ml per minute, equal to about 29% of cardiac output. The hepatic artery blood guarantees the supply of nutrients to the connective tissue (loss of this blood flow can be lethal, inducing necrosis of the main liver structures), and the final outflow is to the hepatic sinusoids, where it combines with the portal blood. Stimulation of the sympathetic nervous system induces vasoconstriction in the hepatic storage areas, especially the large veins, less so in the sinusoids.

In cases of a circulation emergency with an intense sympathetic reaction, a large amount of hepatic blood is put out into the general circulation within 3 or 4 minutes. In a healthy male, this blood volume amounts to about 350 ml.[6]

Liver Sinusoids

The hepatic sinusoids are sheathed by two types of cells:"endothelial cells" and large Kupffer cells (reticulo-endothelial cells), that are able to engulf bacteria and other foreign materials present in blood[4-7]. The hepatic sinusoid walls have a similar endothelium to that of the common capillaries but are much more permeable (due to the presence of much wider pores), so the plasma proteins can pass by diffusion into the liver extravascular spaces almost as easily as fluids. Normally, the pressure in the sinusoids is about 6-8 mmHg, so the proteins can easily spread inside and out. The flow of solutes from the endothelial windows of the sinusoids to Disse's Space (a very small space between the endothelial cells and the liver cells, directly connected with the lymphatic system) is mediated by cytosolic calcium, that modulates the actin-myosin--calmodulin system[8-11].

Hepatic lobules

The functional liver unit is the hepatic lobule. Human liver has from 50 000–100 000 lobules. The hepatic lobule surrounds a central vein whose outflow is to the hepatic veins and finally the vena cava. It is formed by many hepatic cell strands radiating out from the central vein to the lobule periphery, like the rays of a wheel. Each strand is formed by a two-cell thickness and between two cells rows

lay the biliary channels which flow into the terminal biliary ducts, in the septi between adjacent hepatic lobules. The portal venules also run in the septi, carrying blood from the portal vein. From these venules the blood goes to the hepatic sinusoids that are flattened and branched, running between the liver trabeculae hepatic strands. Thus, the liver cells are in continuous contact with the portal venous blood.

With the portal venules in the interlobular septi lie the hepatic arterioles: these arterioles supply arterial blood to septal tissues; some of these flow into the hepatic sinusoids, next to the interlobular septi. In the hepatic vein the blood pressure is normally 0 mmHg, while the portal vein blood pressure is about 9 mmHg. This means that the resistance to blood flow from the portal vein system to the systemic veins through the hepatic sinusoids is normally low.

However, several diseases can increase this resistance, especially liver cirrhosis[12-13]; in this condition the blood flow through the liver is seriously hampered by fibrotic constriction or total obstruction or consumption of the sinusoids.

LIVER MICROCIRCULATION REGULATION

The mechanisms underlying the sinusoidal hepatic blood flow are not yet completely known, but it is important to note that the adrenergic and cholinergic nerves completely control the portal veins and arterioles, modulating sinusoidal blood flow. Moreover, some peptides such as vasoactive intestinal neuropeptide (VIP) and neuropeptide Y (NPY) are present together with noradrenaline and acetylcholine in the nerve endings. In fact, the VIP and NPY, substance P and calcitonin have been identified adjacent to afferent vessels, portal veins and hepatic arterioles, indicating a role in modulating the neurocontrol of the hemodynamics system [14-16]. The Ito Cells in the perisinusoidal space also have an important role, since in the portal venules and hepatic terminal venules these cells collaborate in the control of the sinusoidal blood flow [17-18]. Endothelin I increases the Ito Cells contractility [14]. Another type of cell present here is the Kupffer cell[7] that can temporarily arrest the blood flow through sinusoids.

OXIDATIVE STRESS

Introduction

To fulfil their functions, the cells of aerobic organisms need energy; this energy originates from oxygen as a natural electron acceptor. In physiological conditions many cellular reactions involve oxygen, producing many highly reactive molecules deriving from oxygen itself.

These molecular species include the reactive oxygen species (ROS) or reactive oxygen metabolites (ROM) [19-20]. Anion Superoxide (O^-_2) and the hydroxylic radical (OH) are the most important oxygen-derived free radicals (ROS), but also hydrogen peroxide (H_2O_2) and single oxygen ($^1O_2$), although not chemically definable as free radicals, possess similar features to these latter.

The ROM are essential in the life of all cells, since they superintend all

the redox reactions. Normally, the ROM production is completely under metabolic control, and in fact many blocking systems exist, defined as "the antioxidant biological systems", localized inside and outside the cells, that control the ROMs, delaying or preventing excessive oxidation of the substrates[21-22].

The control mechanisms consist of: (a) neutralizing triggering radicals; (b) preventing inhibitory binding of metallic ions (preventing the generation of triggering radicals); (c) decomposing peroxides to block the reconversion to triggering radicals; (d) blocking the radical cascade to prevent the extraction of other H^+ ions in the lipoperoxidation process.

In disease conditions or simply as a result of certain human lifestyles (cigarette smoking, overeating, physical stress, pollution, etc.) the ROS and other free radicals escape the control of the neutralizing systems and generate toxic phenomena. The set of oxidative alterations induced by ROM (alterations in ion homeostasis, the cytoskeleton, the DNA, loss of the membrane potential, etc.) is denominated "oxidative stress" and is the main cause of cellular ageing[23-24].

Oxidative stress can be defined as the manifestation of exposure of the cells, tissues or organs to an excess of oxidants, especially anion superoxide and ROM. An oxidative stress situation occurs when the production of O_2 and its metabolites exceeds the cell defence and detoxification mechanisms, resulting in problems of gene regulation, signals translation, necrosis, fibrosis and carcinogenesis[25-26]. Normally, the mitochondria are the main resource of reactive oxygen intermediates, since 2-3% of the oxygen consumed is converted to O_2, especially as a result of the auto-oxidation of ubisemiquinone which is able to transfer electrons from complex I and II to complex III [27-28].

Ischaemic/Reperfusion Syndrome increases superoxides production, decreasing the function of complex III[29], tumor necrosis factor alpha[30], and, through an unknown intracellular signal, decreases the function of complex III. Blockage of complex III causes an increased oxygen production due to the auto-oxidation of accumulated ubisemiquinone radicals. The phagocytic cells are an important source of superoxide and other oxidants and can contribute to the reperfusion injury damage[31-32] and to tissue damage during inflammatory processes. The action of nitric oxide (NO) on the endothelium is very important, in that low levels of NO are a relaxant factor, whereas high levels are proinflammatory. The production of NO by macrophages has a bactericide action, but is potentially harmful to the surrounding cells.

Hemorheological alterations during oxidative stress

Oxidative stress plays an important role in the physiopathology of liver damage and fibrosis, in alcohol– or heavy metal-induced diseases. It is also critical to the liver damage caused by ischemia/reperfusion injury[23,31-32] and by sepsis. In chronic liver disease oxidative stress, together with chronic inflammation, can induce hepatocarcinogenesis[35].

Oxidative stress also induces alterations in erythrocyte aggregability and in endothelial adherence[36-37]: the

intercellular interactions between RBC autoaggregation and adhesion of the cells to the endothelium have a very important role in the microcirculation[38-39].

Oxidative stress and Erythrocytes (RBC)

The RBCs are formed only by a membrane and a cytosol, they are denucleated and lack sub cellular organelles. The membrane is highly flexible and deformable and allows these cells to flow even into capillaries with a smaller diameter (3.5 microns) than their own (5-7 microns). The erythrocyte membrane has a peculiar lipid and protid composition; the lipids are responsible for its physical characteristics such as permeability and flexibility, while the proteins, largely glycoproteins, show a 1:1 ratio with the lipids.

Circulating erythrocytes are the cells subjected to the highest oxidative stress and also to oxidant substances produced by the metabolism of some food, drugs, or by bacterial or viral infections. This oxidative stress can induce serious damage due to peroxidation of the membrane lipid matrix and protein polymerisation, leading to lysis or an altered hemoglobin function. In the conversion of hemoglobin to meta-hemoglobin the formation of anion superoxide occurs, which is highly toxic. This can also be brought about by chemical compounds, some drugs, or the action of nitrites derived from food preservatives. The toxicity of anion superoxide is quickly "buffered" by superoxide dysmutase, an oxido-reductase which in RBC is called erythrocuprein. Among non enzymatic detoxification systems, the main substance blocking anion superoxide formation is ascorbic acid and Vit. C.

In physiological conditions, the RBC aggregability induces the formation of rouleaux[38-40]. Normally the blood flow is able to scatter these aggregates before they flow into narrow capillaries, thus ensuring tissue perfusion. In conditions of delayed blood flow (and high plasma viscosity) the RBC aggregability is increased and rouleaux become more plentiful, as well as RBC adhesion to the endothelium. This increases the whole blood viscosity, inducing microcirculatory alterations and the risk of microplugging. Moreover, the ROS present in oxidative stress conditions induce the oxidation of the lipids on the erythrocyte membrane and alterations of their internal and external distribution (resulting in exposure of phosphatidylserine on the cell surface), protein oxidation and disruption, the degradation of surface proteoglycans, hemoglobin oxidation and adherence to the cellular membranes. These alterations have a strong influence on the composition and physical properties of the cell membrane, and modify the RBC aggregability and deformability, increasing RBC adherence to endothelial cells in the capillaries[38-39].

Oxidative stress and platelets

The main functions of the platelets are their mechanical and biochemical actions that play an important role in hemostasis and maintaining the capillaries intact. They are also able to accomplish a process similar

to phagocytosis of foreign particles, as well as the active transport (of serotonin, adrenalin, K^+) and the repair of even minor tissue damage.

When vascular damage occurs, the narrow arterioles and the precapillaries contract, and local vasoconstriction is stimulated by fibrinopeptide B, adrenalin, plasma kinines and serotonin, released by the platelets. In this way, surface phospholipase C is activated, inducing hydrolysis of phosphatidylinositol 4, 5 diphosphate to inositol 4, 5 triphosphate (second messenger) and lipoprotein. This releases calcium ions in the cytosol, causing a contraction of the myosin portion of thromboastenine (contractile cytoskeleton vacuoles), together with the production of serotonin and adenosine-diphosphate (ADP).

Instead, the activation of phospholipase A releases fatty acids (especially arachidonic acid), originating cyclic endoperoxides that, in turn, are converted to thromboxane A_2 (TXA_2), stimulating platelets aggregation and prostaglandins (PGE_2) and prostacyclin (PGI_2), the inhibitors of platelets aggregation.

Oxidative stress, producing free radicals, causes massive changes in the cytoskeleton, inducing irreversible cross-linking between actin and other proteins and thus a decrease in structural peptides. In addition, an important increase in cytosolic calcium can occur, activating transglutaminase that fosters the creation of bridges which stiffen the cytoskeleton, and thus prevent normal platelet functions.

Oxidative Stress and Leukocytes

The leukocytes (WBC) can be subdivided into granulocytes or polymorphonuclear cells (65%) and leukocytes (25%): T, B lymphocytes, plasmocyte precursors, K cells and macrophages precursors, monocytes. The leukocytes have different mechanical properties from the erythrocytes: both can undergo repeated deformation as they pass through the capillaries, but while the RBC have no nuclei or other granules, the leukocytes contain all the cellular components (nucleus, granules, liposomes), that increase the cell rigidity. This degree of rigidity is due to their actin cytoskeleton and actin binding protein and their degree of binding, which allows them to respond to particular situations by taking on long term rigidity or short term elasticity. This property allows the leukocytes to intermittently arrest the capillary blood flow in cases of a fall in perfusion pressure.

One of the main functions of the leukocytes is phagocytosis; when this occurs there is a huge increase in oxygen consumption (respiratory burst), followed by an increased production of anion superoxide and free radicals. At the same time anaerobic glycolysis is stimulated, and phospholipid synthesis, especially of phosphatide acids, phosphatidyl–inositol and phosphatidyl-serine. This could be related to the altered cellular membrane organization required to engulf foreign microorganisms. The oxidative system involved in this microorganisms killing process, characteristic of neutrophil leukocytes, is catalyzed by the NADPH-oxidase enzyme (respiratory burst) localized on the neutrophil membrane in a quiescent state.

Upon stimulation, this system intervenes by activating the neutrophils, a mechanism that involves the mem-

brane receptors, G protein, phospholipase, proteinkinase C and alterations in the interacellular calcium concentration. In this case anion superoxide, that is usually harmful to the cells, is able to mount a strong attack against the bacteria. After stimulation by pro-inflammatory factors, the neutrophils are activated, undergoing a change in their cell body so that their diameter can increase to 10 times its normal size, and thanks to the polymerization of the actin strands, they put out pseudopods. The increased cell rigidity will slow the blood flow in the capillaries. The margination phenomenon of the leukocytes in the bloodstream and their collision with the vessel walls depend on a direct hemodynamic interaction with the RBC in the post-capillary venules. The RBC, in fact, pushes the WBC against the endothelium. An important role in correct endothelial adhesion is played by the "selectins", "integrins" and an immunoglobulin superfamily known as molecules modulating endothelium adhesion[41-43].

Many studies[44-45] have shown an important role of the WBC in disease conditions, especially in conditions of oxidative stress and inflammation, indicating that they are responsible for a higher incidence of vascular disease, since their properties have a strong influence on the microcirculation.

HEMORHEOLOGICAL ALTERATIONS IN LIVER DISEASE

Liver Cirrhosis

The most important hemorheological and functional alterations in the liver microcirculation have been described in the course of cirrhosis[12-13] and ischemic-reperfusion injury syndrome[46].

Cirrhosis is defined as damage to the hepatic acini in zone 3 or zone 1 or both, which is followed by nodular regeneration and the production of fibrous septi. The damage can be such as to induce an impairment of the liver microcirculation, resulting in obstruction and/or blockage of the blood flow. Collagen deposits in Disse's Spaces, with the apparent build-up of an inferior basal membrane in the sinusoidal endothelial cells and the creation of intra-hepatic-porto-hepatic shunts, are the main anatomopathological alterations observed in the liver microcirculation. This picture is also known as hepatic sinusoids "capillarization"[47]. Not only the collagen fibers deposits in the perisinusoidal space, but also the reduction of "endothelial windows", in both number and diameter, will impede the perfusion of the perisinusoidal space, reducing the oxygenation of the remaining hepatocytes and thus triggering a vicious circle that causes the progression of cirrhosis[12]. The structural alterations of the hepatic sinusoids, moreover, contribute to increase the microvasculature resistance, inducing portal hypertension.

An important pathognomonic sign of the evolution of liver disease to cirrhosis is a significant decrease in the blood hemoglobin concentration, resulting in an impairment of liver perfusion and oxygenation of the cirrhotic liver tissue, which was already hypoxic.

Fulminant Hepatitis

In fulminant hepatitis massive bridges of necrotic cells build up

from zone 3 to zone 1, causing major hepatocytic collapse. This situation can induce platelet aggregation in the sinusoids, decreasing or even entirely blocking the blood flow and thus inducing severe hepatic cellular necrosis.

Liver Shock

Physiologically, the liver microcirculation has a self-regulating role that serves to maintain a constant flow through the liver, in response to hemorrhagic shock. In severe cases of shock that overwhelm the self-regulating mechanism, the sinusoidal blood flow diminishes, causing hepatocellular damage in area 3 (centrolobular necrosis) due to an oxygen gradient decrease. Using epi-fluorescence microscopy, an altered sinusoidal perfusion and leukocyte margination have been demonstrated after hemorrhagic shock, together with the production of inflammation mediators (leukotrienes and TNF)[25], which contribute to a further impairment of the liver microcirculation.

The first liver transplantation in humans was performed by Starzl and Coll. in 1963 at the University of Colorado – Denver – USA[48]. In the following years, but especially in the last 19 years, advances in technology, and the use of specially designed drugs (i.e. cyclosporin, tacrolimus, ribavirin, cortisone, etc.) during the postoperative course have stimulated the performance of increasing numbers of liver transplantation procedures, improving disease-free survival. Many liver diseases can only be treated by liver transplantation[49-50]: if no absolute or relative contraindications are present, both children and adults are candidates for liver transplantation if they develop severe, irreversible liver disease that is no longer responsive to medical therapy. Liver transplantation should be considered in patients with end-stage liver disease and life-threatening complications, and with liver failure likely to cause porto-systemic encephalopathy and irreversible damage to the CNS.

In children the most frequent indication for liver transplantation is atresia of the biliary tract, causing progressive alterations of the biliary ducts and cirrhosis, liver failure and death.

Other important indications for liver transplantation in children and adolescents are genetically transmitted diseases with associated progressive liver failure, such as secondary progressive cirrhosis due to an alpha-1-antitrypsin deficit, Wilson's Syndrome, liver failure secondary to Byler's disease, Alagille's disease, Wolman's disease, some forms of glycogenosis, etc.

In adults the most important indications for liver transplantation are chronic active hepatitis of a presumed autoimmune nature, non viral cirrhosis associated with liver failure, end-stage primitive biliary cirrhosis, Caroli's disease, or primitive sclerosing cholangitis with liver failure, irreversible Budd-Chiari Syndrome, primitive hepatocarcinoma. During liver transplantation, hemorheological alterations occur, that vary pre-, peri– and postoperatively.

The hemorheological alterations present **before** transplantation are due to the basal pathology. In cirrhosis, for example, the structural chaos induces increased blood flow resistances and hence decreased tissue

perfusion and hypo-oxygenation, an altered RBC deformability, oxidative stress and the formation of many reactive oxygen substances (ROS).

During transplantation there is generalized ischemia following surgical maneuvers to remove the damaged liver. At the clamped vessels level the blood flow is temporarily arrested, causing an increase in RBC and WBC aggregability and adhesion to the endothelium, platelets activation and hypertension up-stream to the occluded vessel[38,44-45]. This ischemic state must be kept very brief to prevent irreversible damage to the hepatocytes. The hemorheological alterations occurring when these vessels are declamped are vital to the functional recovery of the transplanted liver. T**he ischemic reperfusion injury syndrome** is particularly important[46], as the tissue reoxygenation after prolonged ischemia can compound the damage to the microcirculation induced by the ischemic phase. Capillary alterations and WBC adhesion to the endothelium have been shown in experimental models (rats) after liver transplantation[15].

Later studies revealed an altered relationship between the endothelium and the WBC in the liver sinusoids that was directly correlated to the ischemia and reperfusion time. These studies demonstrated the role of the ROS in fostering WBC adhesion to the sinusoidal endothelium. Activation of the Kuppfer[7] cells, during the post ischemic reperfusion phase, has a direct impact on the adhesion of leukocytes to the transplanted liver endothelium[42]. Calcium and inflammation mediators are also involved. It has been shown that these phenomena are particularly marked in zones 1 and 2 of the liver acini, where there is a greater abundance of Kupffer cells.

An important hemorheological alteration in liver disease and after liver transplantation is a decreased RBC deformability[38]. This phenomenon has been detected using the Cell Transit Analyzer by Erythrocyte Transit Time (ETT)[51]. Before and after liver resection, Adenosine triphosphate (ATP) the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and liver paramaterss (GOT; GPT; gammaGT) have been evaluated. In addition, pre-surgery the green indocyanine level of retention after 15' ($ICGR_{15}$) has been studied, as well as whether RBC deformability alterations were related to this value, and if this evaluation ($ICGR_{15}$) was useful to prevent surgical complications. Green-indocyanine is able to evaluate the total liver blood flow because when injected into the vascular system it can be removed only by the liver. In this way we can calculate the liver blood flow using Fick's formula[52]: Liver Blood Flow = speed of indocyanine removal from the blood/ arterovenous indocyanine difference. The ETT is higher in cirrhotic patients before transplantation than in healthy controls. Moreover, post-surgery complications are higher in patients with high ETT levels on the first, second and third day after transplantation as compared with patients with lower levels [53-54].

ISCHEMIC REPERFUSION (I/R) INJURY.

Organ transplantation (liver, kidney, heart, etc.) raises a number of

important issues regarding microcirculation conditions, hemorheology, tissue oxygenation and how they relate to ischemic-reperfusion injury (I/R), which is the major complication after liver transplantation. I/R injury is a non specific antigen--independent process that can significantly affect the outcome of organ transplantation and constitutes one of the principal risk factors for the development of long-term dysfunction of the transplanted organ.

Paradoxically, reperfusion promotes a series of very complicated pathological events that can injure the tissues[55-62]. The main target of these events is the microcirculation. During I/R injury, xanthine oxidase activity, increased oxygen free radicals (ROS), neutrophil activation and altered adhesion all play important roles. The neutrophils adhere[63] to the microvascular endothelium and induce microcirculatory permeability, which increases transcapillary fluid in the reperfused tissues[58, 64-69].

Additionally, neutrophil adhesion causes post ischemic capillary "no reflow" and has been implicated in the reduced arteriolar sensitivity to vasoactive substances induced by I/R injury[56-58, 70-74].

I/R injury gives rise to a highly complex cascade of phenomena. During ischemia, hypoxia is rapidly induced, rapidly followed by a drop in the intracellular levels of ATP. Aerobic glycolysis is converted to anaerobic glycolysis (piruvate → lactate) resulting in a reduced NADH production. With prolonged ischemia, ATP production drops and energy--dependent functions (Na^+/K^+ dependent ATPase) are arrested, inducing cellular edema and hemoconcentration. Consequently there is a rise in capillary compression, causing reduced capillary perfusion with decreased tissue O_2. The expression of adhesion molecules increases and these in turn increase polymorphonuclear (PMN) leukocytes adhesion to vessel walls. Hypoxia can induce a decreased pH and red blood cells (RBC) deformability; this also contributes to the impairment of capillary perfusion. In such a situation, stasis with microthrombosis will occur and finally, a further deterioration of capillary perfusion.

Ischemia is associated with an important increase in hypoxanthine. When this high concentration of hypoxanthine comes in contact with the oxygen carried by the gush of blood during the early stage of reperfusion, hypoxanthine oxidase gives rise to a high quantity of oxygen derived free radicals (ROS) from the leukocytes, and uric acid (McCord reaction). Via mediators, these ROS could affect the tissue pressure, favouring interstitial edema and microvascular permeability, thus further increasing ROS release from leukocytes. This increase in ROS can directly damage the tissues through the peroxidation of fats. I/R injury can contribute to acute or chronic organ rejection. Leukocytes can contribute to tissue dysfunction during reperfusion. A role in leukocyte adhesion is played by upregulated endothelial ICAM-1 expression during rejection in cardiac, renal, hepatic and corneal allografts[75-81].

This suggests that inflammatory cell infiltration could play a role in transplantation-induced tissue injury. Moreover, I/R injury increases the risk of Delayed Graft Function (DGF) and of acute or chronic rejection. We may

consider that even nowadays, the possible causes of DGF are related only to immunomediated events, but it is possible that non immunological factors such as alterations in the microcirculation could also contribute to I/R injury and to DGF. The high quantity of ROS produced during I/R injury under the effect of xanthine oxidase and a sudden rush of Oxygen and xanthine could play a central role in sustaining the tissue damage via ROS.

After ischemia another important phenomenon is the so-called "capillary no reflow". In fact, a large number of capillaries fail to reperfuse[56-58, 69-70,82-85]. The mechanism remains unclear. One hypothesis is that I/R injury and microvascular thrombus production could induce the "no reflow" phenomenon[86]. However, heparin treatment is unable to restore capillary perfusion after I/R[87]. In any case, miocrovascular thrombosis is unlikely to be present in post ischemic tissues[88-89]. The most credible hypothesis is that post ischemic capillary no reflow is induced by activated neutrophils[90-93]. Following I/R, leukocyte rolling (P-selectin-mediated) occurs, followed by firm adhesion (CD18/ICAM--dependent). These phenomena can induce endothelial cell swelling and finally, capillary no reflow via a reduction of the capillary diameter[94].

CONCLUSIONS

A through knowledge of the hemorheological situation and the microcirculation in liver disease is essential, especially in diseases such as cirrhosis that can completely subvert the liver structure and decrease the blood flow to this organ.

Liver transplantation can often be the only way to save patients with severe liver disease; post-transplant, in these patients we have observed a marked improvement of the peripheral microvasculature, studied by video capillaroscopy and compared with the observations immediately before liver transplantation. The blood, evaluated directly on the liver surface of all patients, also revealed absence of the no reflow phenomenon and an arterial reperfusion level corresponding to physiological values (an increase of approximately 20% of the total liver blood present at the moment of the hepatic artery connection). We found only a reversible, mild cerebral hypoxia that was resolved by increasing the inhaled oxygen. As to the RBC deformability, the situation has been shown to return to normal some days later; this could be explained by resolution of the high levels of bilirubinemia that can induce RBC rigidity. This condition could also explain the low number of functioning peripheral capillaries before transplantation, due to the high blood viscosity induced by RBC rigidity, as well as the increased number of these functioning microvessels after the normalization of bilirubinemia following liver transplantation.

Further studies of transplanted patients are needed to improve the microcirculation conditions following organ transplantation, and especially liver transplantation.

REFERENCES

1. Rappaport SM. The microcirculatory hepatic unit. Microvasc Res 1973; 212:6.
2. Mac Phee P, Schmidt E, *et al.* Organization and flow in the liver microcirculation. Prog Appl Microcirc 1983; 19:52.

3. Guyton A.Trattato di Fisiologia Medica 1987; 388:29.
4. Oda M, Nakamura M, *et al.* Some dynamic aspects of the hepatic microcirculation; demonstration of sinusoidal endothelial fenestrae as a possible regulatory factor. In Intravital Observation of organ Microcirculation, I Ed., Excerpta Medica, Amsterdam 1984:105-138.
5. Oda M; *et al.* Regulatory factors in the hepatic microcirculatory system.Progr. Appl. Microcirc. 1993; 19:25.
6. Sheila Sherlock, Dooley J. Anatomia e funzione del fegato. *In* "Diseases of the Liver and Biliary System" Blackwell Scientific Publications Limited – Oxford Ed. Italiana Momento Medico 1994; 1:1-13.
7. Oda M, Nishida J, *et al.* Relation between sinusoidal endothelial cells and Kupffer cells in hepatic defence mechanism, in Frontiers of Mucosal Immunology, Tsuchiya M, *et al.* (Eds.). Excerpta Medica. Amsterdam 1991; 193-196.
8. Lilly LB, Gollan JL. Ryanodine-induced calcium release from hepatic microsomes and permeabilized hepatocytes Am J Physiol Gastrointest Liver 1995; 268:1017-1024.
9. Robb-Gaspers LD, Thomas AP. Coordination of calcium-signaling by intracellular propagation of calcium waves in the intact liver. J Biol Chem 1995; 270:8102-8107.
10. Nathanson MH, Burgstahler AD, *et al.* Calcium waves are organized among Hepatocytes in the intact organ. Am J Physiol Gastrointest Liver Physiol 1995; 269:167-171.
11. Pinzani M, Failli P, *et al.* Fat-storing cells as liver-specific pericytes. Spatial dynamics of antagonist stimulated intracellular calcium transients. J Clin Invest 1992; 90:642.
12. Oda M, Tsukada N.Abnormalities in the hepatic sinusoid; Pathological basis of self-perpetuation of liver cirrhosis, in Microcirculation in circulatory disorders, Manabe, *et al.* (Ed.). Springer Verlag, Tokyo 1988; 121-133,
13. Sato N, *et al.* Hepatic hemodynamic in patients with chronic hepatitis or cirrhosis as assessed by organ-reflectance spectrophotometry. Gastroenterology 1983; 84:611.
14. Sakamoto M, Ueno T, *et al.* Ito cell contraction in response to endothelin-1 and substance P. Hepathology 1993; 18:978.
15. Gondo K, Uono T, Sakamoto M. The endothelin-1 binding site in rat liver tissue: light and electron-microscopic autoradiographic studies, Gastroenterology 1993; 104:1745.
16. Palmer RM, Moncada S. Vascular endothelial cells synthesise nitric oxide from L-arginine. Nature 1988; 333:664-666.
17. Ito T. Participation of Ito cells in sinusoidals blood flow, *in* Microcirculatio An Update, Tsuchiyam, *et al.* (Eds.). Excerpta Medica Amsterdam 1987; 321-324.
18. Suematsu M, *et al.* Intravital and electron microscopic observation of Ito cells in the rat hepatic microcirculation Microvascular Res. 1993; 46:28.
19. Halliwell B. Free radicals and antioxidants: A personal view. Nutr Rev 1994; 52:253-265.
20. Halliwell B. Antioxidants characterization: Methodology and mechanism. Biochem. Pharmacol 1995; 49:1347-1348.
21. Pahl HL, Baeuerle PA. Oxygen and the control of gene expression. Bioassays 1994; 16:497-502.
22. Meyer M, Schreck R. H2O2 and antioxidants have opposite effects on activation of NF-KB and AP--1 in intact cells: AP-1 as secondary antioxidant response factor. EMBO J 1993; 12:2005-2015.
23. Jaescnhke H. Mechanism of oxidant stress--induced acute tissue injury. Proc Soc Exp Biol Med 1999; 209:104-111.
24. Gutteridge JMC. Biological origin of free radicals and mechanism of antioxidant protection. Chem Biol Interact 1994; 91:133-140.
25. Wong GHW, Elwell JH, *et al.* Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell 1989; 58:923-931.
26. Van Der Viliet A, Bast A. Effect of oxidative stress on receptors and signals transmission. Chem Biol Interact 1992; 85:95-116.
27. Ambrosio G, Zweir JL, *et al.* Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact hearts subjected to ischemia and reflow. J Biol Chem 1993; 268:18 532-18 541.
28. Shulze-Osthoff K, Bakker AC, *et al.* Depletion of the mithocondrial electron transport abrogates the citotoxic and gene-inductive effect of TNF. EMBO J 1993; 12:3905-3104.
29. Gonzales-Flecha B, Cutrin JC. Oxidative stress produced by suprahepatic occlusion and reperfusion. Hepathology 1993; 18:881-889.
30. Shulze-Osthoff K, Bakker AC, *et al.* Citotoxic activity of tumor necrosis factor is mediated by early damage of mithocondrial funtions. J Biol Chem 1992; 267:5317-5323.
31. Koo A, Komatsu H, *et al.* Contribution of "no reflow" phenomenon to hepatic injury following ischemia-reperfusion: evidence for a role for superoxide anion. Hepatology 1992; 15:507-514.
32. Bautista AP, Spitzer JJ. Inhibition of nitric oxide formation in vivo enhances superoxide release by the perfused liver. Am J Physiol 1994; 266:G783-788.
33. Harbrecht BG, Biliar TR, *et al.* Nitric oxide synthesis serves to reduce hepatic damage during acute murine endotoxemia. Crit Care Med 1992; 20:1568-1574.
34. Kuo PC, Livka A. Nitric oxide decreased oxidant mediated hepatocyte injury. J Surg Res 1994; 56:594-600.
35. Hagen TM, Huang S, *et al.* Extensive oxidative damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc Natl Acad Sci USA 1994; 91:12 808-12 812.
36. Baskurt OK, Termiz A, *et al.* Effect of superoxide on red blood cell rheological properties free radical. Biol Med 1998; 24:102-110.
37. Chappey O, Wautier-Pepin MP, *et al.* Adhesion of erythrocytes to endothelium in pathological situation: A review article. Nouv Rev Fr Hemat 1994; 36:281-288.
38. Stoltz JF, Donner M. Hemorheology: importance of erythrocyte aggregation. Clin Hemorheol 1987; 7:3-9.
39. Jones JG. New aspects of red cell aggregation. J Royal Soc Med 1990; 83:663-664.
40. Skalak R, Zarda PR, *et al.* Mechanism of rouleaux formation. Biophys J 1981; 35:771-781.

41. Imhof BA, Dunon D. Leukocyte migration and adhesion. Adv Immunol 1995; 58:345-416.
42. Carlos TM, Harlan MJ. Leukocyte-endothelial adhesion molecules. Blood 1994; 848;2068-2101.
43. Bevilacqua MP, Nelson RM. Selectins. J Clin Invest 1993; 91:379-387.
44. Nash GB, Thomas PR, *et al.* Abnormal flow properties of white blood cells in patients with severe ischemia of the leg. Br Med J 1988; 296:1699--1701.
45. Grau AJ, Berger E. Granulocyte adhesion, deformability and superoxide formation in acute stroke. Stroke 1992; 22:33-39.
46. Jaeschke H. Reactive oxygen and ischemia/reperfusion injury of the liver. Chem Biol Interact 1991; 79:115-136.
47. Schaffner F, Popper H. Capillarization of hepatic sinusoid in man. Gastroenterology 1963; 44:234.
48. Schmid R. Trapianto di fegato in Dionigi Basi teoriche e Clinica Chirurgica 2006.
49. Busutill RW. Liver transplantation today. Ann Intern Med 1986; 104:377.
50. Maddrey WC. Transplantation of the liver New York Elsevier 1988.
51. Katsuhiko H, Shoji K. Changes in erythrocyte deformability after liver resection foe hepatocarcinoma associated with chronic liver disease. World J Surg 1999; 23:85-90.
52. Guyton A. Trattato di Fisiologia Medica 1987; 966:17-35.
53. Shiga T, Maeda N. Erythrocyte rheology. Crit Rev Oncol Hemat 1990; 10:9.
54. Langenfield JE, Livingston DH. Red blood cell deformability is an early indicator of infection. Surgery 1991; 110:398.
55. Cicco G, Panzera PC, Catalano G, Memeo V. Microcirculation and reperfusion injury in organ transplantation. *In:* Oxygen Transport to Tissue XXVI edr P. Okunjeff Springer New York USA AEMB 2005; 566;363-373.
56. Jerome SN, *et al.* CD-18 dependent adherence reactions play an important role in the development of the no reflow phenomenon. Am J Physiol 1993; 264:479-283.
57. Parks DA, Granger DN. Contributions of ischemia and reperfusion to mucosal lesion formation. Am J Physiol 1986; 250:749-753.
58. Carden DL, Smith JK, *et al.* Neutrophils mediated microvascular dysfunction in postischemic canine skeletal muscle: role of granulocyte adherence. Circ Res 1990; 66:1436-1444.
59. Perry MA, Wodhwa SS. Gradual reintroduction of oxygen reduces perfusion injury in cat stomach. Am J Physiol 1988; 254:366-372.
60. Korthuis RJ, Smith JK. Hypoxic reperfusion attenuates post ischemic microvascular injury. Am J Physiol 1989; 256:315-319.
61. Dahlback LO, Rais O. Morphological changes in striated muscle following ischemia: immediate post ischemic phase. Acta Chir Scand 1966; 131:430-440.
62. Morris JB, *et al.* The protection from postischemic injury by xanthine oxidase inhibition: Blockage of free radical generation or purine salvage. Gastroenterology 1987; 92:1542.
63. Dunn ND, *et al.* Leukocyte/endothelial cell adhesion and ischemia reperfusion injury in Clinically applied Microcirculation Research edr Barker J. CRC Press Inc Boca Raton FL 1995; 75-96.
64. Adkins WK, Taylor E. Role of xanthine oxidase and neutrophils in ischemia-reperfusion injury in rabbit lung. J Appl Physiol 1990; 69:2012-2018.
65. Hermandez LA, Grisham MB, *et al.* Role of neutrophils in ischemia/reperfusion induced microvascular injury. Am J Physiol 1987; 253:699-703.
66. Bishop MJ, *et al.* Antibody against neutrophils adhesion improves reperfusion and limits alveolar infiltrate following unilateral pulmonary artery occlusion. J Surg Res 1992; 52:199-204.
67. Horgan MJ, Ge M, Gu J, Rothlein R, Malik B. Role of ICAM-1 in neutrophil mediated lung vascular injury after occlusion and reperfusion. Am J Physiol 1991; 261:1578-1584.
68. Horgan MJ, Malik AB. Antibody against leukocyte integrin (CD 18) prevents reperfusion induced lung vascular injury. Am J Physiol 1990; 259:315-319.
69. Vedder NB, Winn RK, *et al.* Inhibition of leukocyte adherence by anti-CD 18 monoclonal antibody attenuates reperfusion injury in the rabbit ear proc. Natl Acad Sci USA 1990; 87:2643-2646.
70. Jerome SN, Dore M, *et al.* P-Selectin and ICAM-1 adherence reactions: role in the genesis of postischemic no reflow. Am J Physiol 1994; 266:1316--1321.
71. Mori E, Del Zoppo GJ, Chambers JD, Copeland BR, Arfors E. Inhibition of polymorphonuclear no-reflow after local cerebral ischemia in baboons. Stroke 1992; 23:712-718.
72. Ma XL, Tsao PS, Lefer AM. Antibody to CD 18 extends endothelial and cardiac protective effects in myocardial ischemia and reperfusion. J Clin Invest 1991; 88:1237-1243.
73. Ma XL, Lefer AM, Rothlein R. Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule-1 in myocardial ischemia and reperfusion. Circulation 1992; 86:937-946.
74. Weyrich AS, Ma SY, Lafer K. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. J Clin Invest 1993; 91:2620-2629.
75. Adams DH, Hubscher SG, Shaw J. Intercellular adhesion molecule-1 on liver allografts during rejection. Lancet 1989; 2(8672):1122-1125.
76. Adams DH, Wang LF, Burnett D. Neutrophil activation an important cause of tissue damage during liver rejection? Transplantation 1990; 50(1):86-91.
77. Elver VM, Elmer SG, Pavilack MA. Intercellular adhesion molecule-1 in human corneal endothelium. Am J Pathol 1991; 138(3):525-536.
78. Hubscher SG, Adams DH. ICAM-1 expression in normal liver. J Clin Pathol 1991; 44(5):438-439.
79. Omura T, Ishikura H, Nakajima Y. The expression of FA-1 ICAM-1 in liver transplantation in rats. Transpl Proceed 1992; 24:1618.
80. Sedmak DD, Orasz CG. The role of vascular endothelial cells in transplantation in rats. Transplantation Proceedings 1992; 24:1237.
81. Takei Y, Marzi I, Gao W, Gores J. Leukocyte adhesion and cell death following orthotopic liver transplantation in the rat. Transplantation 1991; 51(5):959-965.
82. Lehr HA, Gulhman A, Nolte D, Keppler D. Leukotrienes as mediators in ischemia reperfusion injury in a microcirculation model in the Hamster. J Clin Invest 1991; 87(6):2036-2041.

83. Ames III A, Wright RL, Kowada M. Cerebral ischemia II the no reflow phenomenon. Am J Pathol 1968; 52(2):437-453.
84. Schmid-Schonbein, GW. Capillary plugging by granulocytes and the no reflow phenomenon in the microcirculation. Fed Proceed 1987; 46(7):2397-2401.
85. Cicco G. Hemorheology, reperfusion injury and organ transplantation 12 ^ ECCH Sofia (Bulgaria) A.B. RTI 2003; 1:64.
86. Quinones-Baldrich WJ, Chervu A, Hermandez JJ. Skeletal muscle function after ischemia "no reflow" versus reperfusion injury. J Surg Res 1991; 51(1):5-12.
87. Strock PE, Majno GM. Vascular responses to experimental tourniquet Ischemia. Surg Gynec Obstetr 1969; 129(2):309-318.
88. Harman JW. The significance of local vascular phenomena in the production of ischemia necrosis in skeletal muscle. Am J Pathol 1948; 24(1): 625.642.
89. Bagge U, Amundson B. White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiol Scand 1980; 108(2):159-163.
90. Barrosa-Aranda J, Schmid-Schonbein GW, Zweifach BW. Granulocytes and no reflow phenomenon in irreversible hemorrhagic shock. Circ Res15 1988; 63(2):437-447.
91. Engler RI, Schmid-Schonbein GW, Pavalec RS. Leucocyte capillary plugging in myocardial ischemia and reperfusion in the dog. Am J Pathol 1983; 111(1):98-111.
92. Jerome SN, Akimitsu T, Korthuis RI. leucocytes adhesion, edema and the development of post ischemia capillary no reflow. Am J Physiol 1994; 267(4):1329-1336.
93. Jerome SN, Akimitsu T, Korthuis RJ. Ischemic preconditioning alternates capillary alternates capillary no reflow induced by prolonged ischemia and reperfusion. Am J Physiol 1995; 268(2):2063--2067.
94. Mazzoni MC, Intaglietta M, Arfors KE. Luminal narrowing and endothelial cell swelling in skeletal muscle capillaries during hemorrhagic shock. Circ Shock 1989; 29(1):27-39.

ASSOCIATIONS OF CIRCULATING TNFALPHA AND IL-18 WITH MYOCARDIAL INFARCTION AND CARDIOVASCULAR RISK MARKERS: THE GLASGOW MYOCARDIAL INFARCTION STUDY

(ARTIGO ORIGINAL)

Welsh P, Woodward M, Rumley A, Lowe G.

Background: There are a lack of data on the associations of circulating levels of TNFalpha and IL-18 with myocardial infarction (MI), and on the extent of confounding by classical and inflammatory risk markers. **Methods:** We measured TNFalpha and IL-18 in plasma from 446 MI cases and 477 age- and sex-matched controls from North Glasgow. **Results:** TNFalpha and IL-18 were elevated in cases compared to controls (TNFalpha medians 0.99 pg/ml [interquartile range 0.65-1.64 pg/ml] versus 0.77 pg/ml [0.52-1.22 pg/ml], p<0.0001; IL-18 medians 287 pg/ml [212-404 pg/ml] versus 271 pg/ml [200-373 pg/ml], p=0.01). IL-18 was moderately associated with HDL cholesterol r=-0.22, triglycerides r=0.16, and BMI r=0.14 (p for all < or =0.003) in the control population, but not among cases. TNFalpha had few associations with classical risk factors among cases or controls. TNFalpha had a significant association with MI: odds ratio (OR) 1.66 (95% confidence interval; 1.10-2.50), comparing extreme thirds after adjusting for classical risk factors, which was reduced on further adjustment for other inflammatory markers (OR 1.47; 0.91-2.37). IL-18 showed no association by thirds after adjustment for classical risk factors (OR 1.07; 0.70-1.62). **Conclusions:** Circulating levels of IL-18 and TNFalpha were elevated in those with previous MI, but only TNFalpha retained an association after adjustment for classical risk factors. Independently elevated TNFalpha among those with previous MI may reflect cardiac expression of TNFalpha in ongoing myocardial remodeling. [**Cytokine 2009; 47(2): 143-147]**

PMID: 19581111

MICROCIRCULATORY EFFECTS OF CHANGING BLOOD HEMOGLOBIN OXYGEN AFFINITY DURING HEMORRHAGIC SHOCK RESUSCITATION IN AN EXPERIMENTAL MODEL

(ARTIGO ORIGINAL)

Villela NR, Cabrales P, Tsai AG, Intaglietta M.

Microvascular responses to blood volume restitution using red blood cells (RBCs) with modified hemoglobin (Hb) oxygen affinity were studied in the hamster window chamber model during resuscitation from hemorrhagic shock. Allosteric effectors inositol hexaphosphate and 5-hydroxymethyl-2-furfural were introduced into the RBCs by electroporation to decrease and increase Hb-oxygen affinity. In vitro P50s (partial pressure of oxygen at 50% Hb saturation) were modified to 10 and 50 mmHg (normal P50, 32 mmHg). Awake hamsters were subjected to hemorrhage of 50% of blood volume, followed by a shock period of 1 h, and then resuscitated with 25% blood volume with high or low P50 RBCs (hematocrit, 50%). After resuscitation, base excess was significantly lower than baseline in the high-P50 RBC group (HP50; 0.3 +/- 2 vs. 5.0 +/- 1.7 mM) and MAP was lower than baseline in the low-P50 RBC group (LP50; 93 +/- 6 vs. 109 +/- 6 mM). Arteriolar diameter and flow were significantly lower in the HP50. Functional capillary density in the HP50 was significantly lower than LP50 at 60 and 90 min after resuscitation. There was no significantly difference in arteriolar PO2. Tissue PO2, venular PO2, and oxygen delivery were higher in LP50 than in HP50. There was no significant difference in oxygen extraction. Oxygen extraction ratio (oxygen extraction/oxygen delivery) x 100 was significantly higher in HP50 than in LP50. These results suggest that lowering blood P50 in resuscitation provides improved microvascular function in comparison with higher P50. **[Shock 2009; 31(6):645-652]**

PMID: 18948853

META-ANALYSIS: RETINAL VESSEL CALIBER AND RISK FOR CORONARY HEART DISEASE

(ARTIGO ORIGINAL)

McGeechan K, Liew G, Macaskill P, Irwig L, Klein R, Klein BE, Wang JJ, Mitchell P, Vingerling JR, Dejong PT, Witteman JC, Breteler MM, Shaw J, Zimmet P, Wong TY.

Background: Retinal vessel caliber may be a novel marker of coronary heart disease (CHD) risk. However, the sex-specific effect, magnitude of association, and effect independent of traditional CHD disease risk factors remain unclear. PURPOSE: To determine the association between retinal vessel caliber and risk for CHD. **Data sources:** Relevant studies in any language identified through MEDLINE (1950 to June 2009) and EMBASE (1950 to June 2009) databases. **Study selection:** Studies were included if they examined a general population, measured retinal vessel caliber from retinal photographs, and documented CHD risk factors and incident CHD events. **Data extraction:** 6 population-based prospective cohort studies provided data for individual participant meta-analysis. **Data synthesis:** Proportional hazards models, adjusted for traditional CHD risk factors, were constructed for retinal vessel caliber and incident CHD in women and men. Among 22,159 participants who were free of CHD and followed for 5 to 14 years, 2219 (10.0%) incident CHD events occurred. Retinal vessel caliber changes (wider venules and narrower arterioles) were each associated with an increased risk for CHD in women (pooled multivariable-adjusted hazard ratios, 1.16 [95% CI, 1.06 to 1.26] per 20-microm increase in venular caliber and 1.17 [CI, 1.07 to 1.28] per 20-microm decrease in arteriolar caliber) but not in men (1.02 [CI, 0.94 to 1.10] per 20-microm increase in venular caliber and 1.02 [CI, 0.95 to 1.10] per 20-microm decrease in arteriolar caliber). Women without hypertension or diabetes had higher hazard ratios. **Limitation:** Error in the measurement of retinal vessel caliber and Framingham variables was not taken into account. **Conclusion:** Retinal vessel caliber changes were independently associated with an increased risk for CHD events in women. **[Ann Intern Med 2009; 151(6):404-413].**

PMID: 19755365

WALL-TO-LUMEN RATIO OF RETINAL ARTERIOLES AS A TOOL TO ASSESS VASCULAR CHANGES

(ARTIGO DE REVISÃO)

Ritt M, Schmieder RE

The retina offers a beautiful and unique opportunity tovisualize and examine the body's microvasculature safely, repeatedly, quickly, and noninvasively in vivo. Retinal arterioles appear to undergo similar changes as cerebral and peripheral arterioles in hypertension, indicating that retinal arteriolar abnormalities mirror structural and functional microvascular changes elsewhere in end-organ tissues.1 Since the pioneering work by Keith et al5 in 1939, several studies have confirmed the prognostic significance of retinal vascular abnormalities on mortality attributed to a cardiovascular cause. However, although there is solid evidence for the prognostic significance of advanced retinopathy, the evidence of a prognostic impact of early retinal vascular abnormalities on cardiovascular risk stratification is less well established. It was suggested that methodological issues might be the cause for the lack of a solid evidence that early retinal vascular abnormalities are closely linked to cardiovascular risk. Therefore, much research effort over the last decade has focused on the development of new methodological approaches to enable more precise and reliable detection and evaluation of early retinal vascular abnormalities in hypertensive patients. A new approach focuses on retinal arteriolar structural parameters by using scanning laser Doppler flowmetry (SLDF) with automatic full-field perfusion imaging analyses (AFFPIAs).This approach allows the assessment of both the outer diameter (OD) and inner diameter (ID) of retinal arterioles in vivo and, thus, analyzes vascular remodeling of retinal arterioles by calculating wall:lumen ratio, wall thickness, and wall cross-sectional area (volume of wall per unit of length) of retinal arterioles. These methods do not need to determine diameter of retinal venules, which are also subject to changes in cardiovascular disease. This review introduces and describes this new methodology, explains the improved power of measuring retinal vascular changes, and discusses our recent findings using this tool. **[Hypertension 2009; 54(2):384-387]**

PMID: 19451413

CIÊNCIA NOS ALPES

15TH CONFERENCE OF THE EUROPEAN SOCIETY FOR CLINICAL HEMORHEOLOGY AND MICROCIRCULATION

Fig. 1 – Local da reunião

Fig. 2 – O Professor Friedrich Jung recebendo o Fahraeus Award 2009,apresentado pelo anterior galardoado, Professor Michael Rampling

Entre 28 de Junho e 1 de Julho um dos mais conhecidos "resorts" da Suíça, o de Pontresina / St. Moritz (Fig. 1), recebeu o importante evento científico "*15th Conference of the European Society for Clinical Hemorheology and Microcirculation (ESCHM)*", este ano organizado e presidido pelo Professor Walter H. Reinhart.

A reunião incluiu conferências plenárias, simpósios, apresentações orais livres e sessões de "posters" sobre aspectos diversos de Hemorreologia e Microcirculação.

Foram conferencistas Sandro Forconi, Shu Chien, Tommaso Gori, Herb Meiselman and Gerard Nash. Com a *altitude* como tema, assistimos também a uma conferência muito especial com cariz mais de "Curiosidade" sobre importantes alterações fisiológicas que ocorrem devido à alta altitude. Esta interessante conferência foi-nos apresentada por Peter Bärtsch da Universidade de Heidelberg. Por último, o presidente da *European Society for Clinical Hemorheology and Microcirculation*, Friedrich Jung, recebeu o prémio Fahraeus deste ano (Fig. 2).

Mais uma vez o grupo de trabalho de uma das pessoas responsáveis pelo estudo da Hemorreologia em Portugal, Carlota Saldanha, esteve presente para participar e apresentar parte do trabalho (em duas comunicações

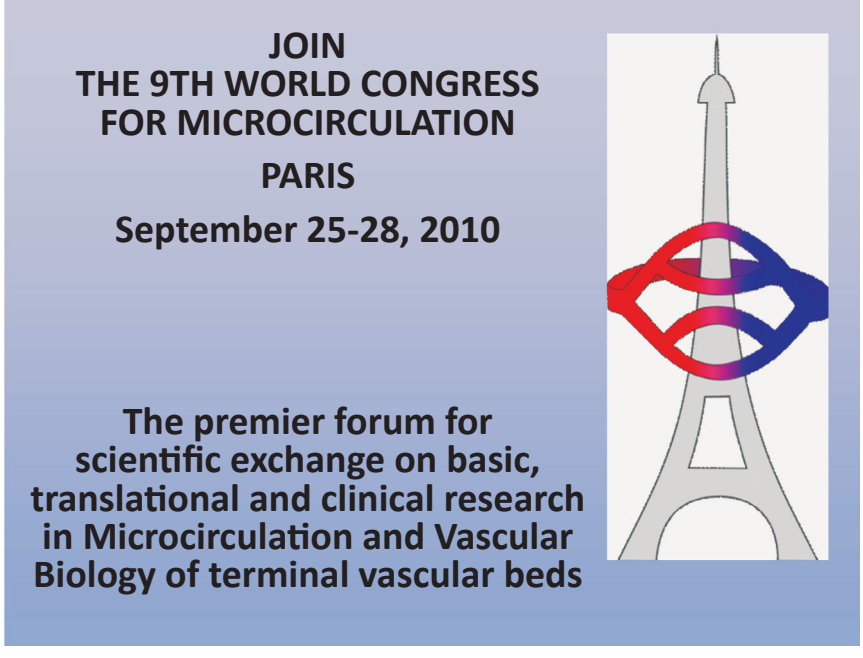
orais) que tem vindo a ser desenvolvido na Unidade de Biologia da Microcirculação e Inflamação (UBiMI), onde é a investigadora responsável, no Instituto de Medicina Molecular (IMM), Faculdade de Medicina de Lisboa.

Carlota Saldanha teve a seu cargo, juntamente com a cientista russa I. Tikhomirova, a organização e moderação de um dos simpósios ("*Role of activation molecular signaling pathways in erythrocyte rheologic properties*"), no qual foram apresentadas as duas comunicações,intituladas "*Fibrinogen and erythrocyte membrane interaction: potential binding sites evaluation*" e "*Erythrocyte band 3 Protein and Hemorheological Properties*". A primeira apresentação, a cargo de Sofia de Oliveira, relatou alguns dos resultados obtidos por si obtidos durante o último ano e meio do projecto "*Fibrinogénio e transducção de sinal na microcirculação–implicação em inflamação e doenças cardiovasculares*" (PTDC/SAU-OSM/73449/2006, de que é orientadora Carlota Saldanha). A segunda intervenção teve por finalidade resumir os estudos da proteína banda 3, desenvolvidos por Carlota Saldanha nos últimos 5 anos.

A próxima (16.ª) Conferência Europeia terá lugar em Munique, em 2011estando a respectiva organização a cargo da investigadora búlgara Nadia Antonova.

Sofia de Oliveira

9.º CONGRESSO MUNDIAL SOBRE MICROCIRCULAÇÃO/2010



Realiza-se em Paris, de 25 a 28 de Setembro de 2010, a próxima grande reunião sobre Microcirculação. Para mais informações consulte o número anterior do Boletim ou o endereço seguinte: micro.2010@lrb.aphp.fr (presidente Eric Vicaut)

CONGRESSO MEET 2010 – MULTIDISCIPLINARY EUROPEAN ENDOVASCULAR THERAPY

Está marcada para Marselha, de 17 a 20 de Junho de 2010, a tradicional reunião MEET. Para mais informações consulte o anúncio anexo e endereços aí referidos.

REGRAS DE PUBLICAÇÃO / INSTRUCTIONS TO AUTHORS

CONVITE

A Sociedade Portuguesa de Hemorreologia e Microcirculação (SPHM) aceita para publicação no seu BOLETIM artigos de curta extensão. O Boletim é editado quatro vezes por ano em formato de papel e electrónico (www.hemorreologia.com), sendo), sendo distribuído gratuitamente aos sócios, individualidades e instituições científicas e culturais.

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.

4. Os manuscritos com o pedido de publicação devem ser enviados por *e-mail* ao Editor (carlotasaldanha@fm.ul.pt).

– Comunicações Originais (artigos curtos) – Os textos serão considerado para publicação rápida, com a seguinte estrutura: Sumário (50-70 palavras), Introdução, Material e Métodos, Resultados, Discussão e Conclusões. O(s) autor(es) são estimulados a englobar em conjunto os resultados, discussão e conclusões.
(Extensão máxima do texto: 5 a 6 páginas a um espaço (letra de corpo 11), incluindo figuras tabelas e quadros(e respectivas legendas),agradecimentos e até 30 referências bibliográficas).

– Artigos de Revisão – O BOLETIM terá a maior satisfação em acolher curtas revisões sobre assuntos de particular interesse, no âmbito da Hemorreologia, Microcirculação ou assuntos de âmbito médico ou de outras áreas científicas afins, que sejam submetidos directamente para publicação ou mediante convite especial do Editor.
(Extensão máxima do texto:8 a 10 páginas (letra de corpo 11) incluindo figuras, tabelas, quadros, fotos (e respectivas legendas), agradecimentos e até 60 referências bibliográficas).

INVITATION

The Portuguese Society on Hemorheology and Microcirculation (Sociedade Portuguesa de Hemorreologia e Microcirculação, SPHM) is pleased to welcome short papers for publication in its BOLETIM. This publication, in paper and online (www.hemorreologia.com), is distributed four times a year free of charge to the members of the Society.

INSTRUCTIONS

1. All submitted manuscripts are subjected to editorial review and approval. The decision to publish is dependent on the scientific and cultural merit of the papers.

2. Only contributions prepared and submitted as optic version (*PDF* or *Microsoft Word*), will be accepted.

3. Texts must be written in Portuguese or in English.

4. All scientific contributions, including manuscript submission and further correspondence should be addressed by *email* to the Editor (carlotasaldanha@fm.ul.pt)

– Original Communications – Manuscripts may be considered for rapid processing as short communications. All manuscripts should be arranged in the following sections: Abstract (50-70 words), Introduction, Material and Methods, Results, Discussion, Acknowledgements and References. The author(s) may combine some of the sections normally included in a full paper, namely the results, discussion and conclusions.
(Maximum communication length – 5-6 single spaced typed pages, including figures, tables, legends, acknowledgments and up to 30 references).

– Short Reviews – The BOLETIM will publish reviews on subjects of particular interest in its field, either following a special invitation or a submission by the author, and in the latter case only after approval by an Editorial Board member. Further information can be obtained from the editor.
(Maximum review length – 8-10 full pages, including figures, tables, photos, legends, acknowledgments and up to 60 references)