

# Vascular Wall and Endothelium

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**2008**

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Lisboa – Portugal

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Actas de Bioquímica  
Apartado 4098  
1500-001 Lisboa – Portugal

#### **Subscription Information**

Subscription price is \$25.00 (twenty five US dollars) or €25,00 (twenty five euros) per volume. An additional charge of €5,00 (five US dollars) per volume is requested for post delivery outside Portugal. Payment should accompany all orders. Correspondence concerning subscription should be addressed to the mailing address above.

ISBN: 972-590-076-6

**VASCULAR WALL AND ENDOTHELIUM**

LISBON (PORTUGAL),  
SEPTEMBER 14, 2007

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Fundação Merck Sharp e Dohme  
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## **VASCULAR WALL AND ENDOTHELIUM**

Proceedings of the Symposium on Vascular Wall and Endothelium

**(7th Advanced Course on Applied Biochemistry)**

Held in Lisbon, September 14, 2007.

Organized by the Institute of Biopatologia Química, Faculdade de Medicina,  
and Unidade de Biopatologia Vasculuar do Instituto de Medicina Molecular,  
Universidade de Lisboa

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### **Published by**

Instituto de Biopatologia Química

Faculdade de Medicina

Universidade de Lisboa

Agradecimentos à Fundação Merck Sharp e Dhome  
pelo apoio financeiro concedido à realização e publicação  
dos textos do 7.º Curso Avançado de Bioquímica Aplicada.



F U N D A C ã O  
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Printed in Portugal

## ÍNDICE

## LECTURES

<b>Introduction to endothelial cells mechanobiology</b> .....	1
<i>Kadi A, Decot V, Muller S, Menu P, Ouyan T, Traore M, Bensoussan D, Stoltz JF</i>	
<b>The role of Dll4 in the regulation of arteriogenesis and angiogenesis</b> .....	15
<i>Alexandre Trindade, António Duarte</i>	
<b>Leukemia cells and the bone marrow endothelium</b> .....	25
<i>João T Barata</i>	
<b>Computational hemorheology: progress in blood coagulation modelling</b> .....	31
<i>Adélia Sequeira, Tomáš Bodnár</i>	
<b>Mechanisms that regulate cell turnover or transformation in the bone marrow microenvironment</b> .....	43
<i>Catarina Osório, Ana Sofia Cachaço, Sergio Dias</i>	
<b>Non-neuronal cholinergic mechanisms in red blood cell</b> .....	49
<i>C Saldanha</i>	

## POSTERS

<b>Angiogenesis in breast carcinomas with different expression profiles</b> .....	59
<i>Nair Lopes, Bárbara Sousa, Daniella Vieira, Fernanda Milanezi, Fernando Schmitt</i>	
<b>Erythrocyte damage/aging/removal are enhanced in both mother and fetus, in preeclampsia</b> .....	63
<i>Cristina Catarino, Irene Rebelo, Luís Belo, Petronila Rocha-Pereira, Susana Rocha, Elisabeth Bayer Castro, Belmiro Patrício, Alexandre Quintanilha, Alice Santos-Silva</i>	

<b>Endothelial function in newborn infants from preeclamptic pregnancies .....</b>	<b>69</b>
<i>Cristina Catarino, Irene Rebelo, Luís Belo, Susana Rocha, Elisabeth Bayer Castro, Belmiro Patrício, Alexandre Quintanilha, Alice Santos-Silva</i>	
<b>Altered erythrocyte membrane band 3 profile in chronic renal failure patients under haemodialysis .....</b>	<b>73</b>
<i>Elísio Costa, Susana Rocha, Petronila Rocha-Pereira, Elisabeth Castro, Flávio Reis, Frederico Teixeira, Vasco Miranda, Maria do Sameiro Faria, Alfredo Loureiro, Alexandre Quintanilha, Luís Belo, Alice Santos-Silva</i>	
<b>Fibrinolytic activity and vascular access in chronic renal failure patients under haemodialysis .....</b>	<b>79</b>
<i>Elísio Costa, Susana Rocha, Petronila Rocha-Pereira, Elisabeth Castro, Flávio Reis, Frederico Teixeira, Vasco Miranda, Maria do Sameiro Faria, Alfredo Loureiro, Alexandre Quintanilha, Luís Belo, Alice Santos-Silva</i>	
<b>Effects of Acetylcholine on NO translocation in abnormal and manipulated red blood cells.....</b>	<b>83</b>
<i>FA Carvalho, JP Almeida, J Guerra, J Ducla Soares, JA Albino, C Moreira, JM Braz Nogueira, AV Maria, H Luz Rodrigues, L Caeiro, JM Ferro, J Martins e Silva, C Saldanha</i>	
<b>Identification of the linkage between g proteins and erythrocyte protein band 3 .....</b>	<b>89</b>
<i>FA Carvalho, J Martins e Silva, C Saldanha</i>	
<b>Oxidative stress, endothelial dysfunction and vascular disease on obesity and penile erectile dysfunction .....</b>	<b>95</b>
<i>FA Carvalho, J Martin-Martins, S do Vale, J Martins e Silva, C Saldanha</i>	
<b>Kdr – a nuclear signalling protein.....</b>	<b>101</b>
<i>Inês Domingues, Helena Pina, Zhenping Zhu, Yan Wu, Sérgio Dias, Susana Constantino Rosa Santos</i>	
<b>Vasculature activation and tumor re-growth after radiotherapy.....</b>	<b>105</b>
<i>Inês Sofia Vala, Antonieta Ferreira, Isabel Monteiro Grillo, Susana Constantino Rosa Santos</i>	
<b>The modulation of cyclic nucleotide levels and PKC activity by acetylcholinesterase effectors in human erythrocytes.....</b>	<b>111</b>
<i>JP Almeida, FA Carvalho, J Martins-Silva, C Saldanha</i>	
<b>Signaling transduction pathways implicated in NO production in endothelial cells treated with a selective <math>\beta_1</math>-adrenergic receptor antagonist .....</b>	<b>115</b>
<i>A Kadi, N de Isla, P Lacolley, P Menu, JF Stoltz</i>	

<b>Hemorheologic parameters in animal models of hyper and hypocholesterolemia and hypertension.....</b>	<b>119</b>
<i>AS Silva-Herdade, C Saldanha</i>	
<b>Haemorheological changes during recombinant human erythropoietin therapy in a rat model of renal failure induced by partial nephrectomy .....</b>	<b>125</b>
<i>Elísio Costa, Flávio Reis, Petronila Rocha-Pereira, Sofia Baptista, André Dias, Susana Rocha, Elisabeth Castro, Vasco Miranda, Maria Sameiro Faria, Alfredo Loureiro, Edite Teixeira de Lemos, Belmiro Parada, Arnaldo Figueiredo, Alexandre Quintanilha, Frederico Teixeira, Luís Belo, Alice Santos-Silva</i>	
<b>Advances in Computational Hemorheology.....</b>	<b>131</b>
<i>Adélia Sequeira, Abdel Monim Artoli, Euripides Sellountos, João Janela</i>	
<b>Coupling multiscale fluid-structure interaction models for blood flow simulations ....</b>	<b>137</b>
<i>Alexandra Moura</i>	
<b>Shear-thinning dependent mechanisms of leukocyte recruitment to the endothelial wall... </b>	<b>143</b>
<i>AM Artoli, A Sequeira, AS Silva-Herdade, C Saldanha</i>	
<b>Instructions to Authors.....</b>	<b>149</b>

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

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### ABSTRACT

The aim of this work was to study the effect of recombinant human erythropoietin (rhEPO) therapy on haemorheological parameters, by using a rat model of chronic renal failure (CRF) induced by partial ( $3/4$ ) nephrectomy.

The study used adult male Wistar rats and was performed in three groups: a control one (n=6) and in two groups with induced chronic renal failure (n=9), being one of them submitted to rhEPO therapy (n=4). Blood samples from the control group were collected at the beginning and at the end of the experimental procedure and from CRF rats at 3, 5, 8, 12 and 15 weeks after surgical partial nephrectomy. Haemorheology and renal function were evaluated.

Three weeks after the  $3/4$  nephrectomy, a statistically significant increase in serum urea and creatinine concentrations were found. This increase in renal function markers remained high along the 12 weeks of experimental procedure.

Comparing with controls, rhEPO treated rat have showed a statistically significant progressive increase in haemoglobin (Hb), haematocrit (Ht), red blood cells (RBC) count, mean cell volume

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

Partial nephrectomy seems to be a suitable methodology to induce CRF in rats and to study erythropoiesis biology. The rhEPO therapy is associated with an increased erythropoietic stimulation and a decrease in platelet count.

Key-Words: rhEPO, Erythropoietin, renal failure, rat model, Haemorheology.

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## INTRODUCTION

Erythropoietin (EPO) is a glycoprotein hormone produced by kidneys, which regulates the proliferation, differentiation and maturation of erythroid cells. Chronic renal failure (CRF) patients develop anaemia due, mainly, to the low production of EPO by kidneys. To treat this anaemia, recombinant human EPO (rhEPO) therapy is currently used in these patients<sup>1-3</sup>.

The aim of this work was to study the effect of rhEPO therapy on haemorheological parameters, by using a rat model of CRF induced by partial (3/4) nephrectomy.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (Charles River Lab. Inc.), 380-400g, were maintained in an air conditioned room, subjected to 12-h dark/light cycles and given standard laboratory rat chow (IPM-R20, Letica) and free access to tap water.

### Study design

The study was performed in three groups: a control one (n=6) and in two groups with induced CRF (n=9), being one of them submitted to rhEPO therapy (n=4).

CRF was induced by a two-stage (3/4) nephrectomy: first, about half of the left kidney was removed by left flank incision and, one week later, the right kidney was removed through a right lateral flank incision<sup>3-8</sup>.

After a three weeks stabilization period, four animals start rhEPO therapy (Recormon, Roche Pharmaceuticals, Auckland, New Zealand) in a dose of 50 IU/Kg/week, during 12 weeks.

### Blood samples and Assays

Blood samples from the control group were collected at the beginning and at the end of the experimental procedure, and from CRF rats at 3, 5, 8, 12 and 15 weeks after surgical partial nephrectomy.

In all animals (controls and CRF) renal function was evaluated by determination of serum levels of urea and creatinine. Red blood cells (RBC) count, haema-

tocrit (Ht), haemoglobin concentration (Hb), haematimetric indices [mean cell volume (MCV) and mean cell Hb (MCH)], red cell distribution width (RDW), white blood cells (WBC) and reticulocyte counts were also evaluated by using a blood cell counter.

### Data Analysis

For statistical analysis, we used the Statistical Package for Social Sciences, version 14.0. Results are presented as means  $\pm$  SEM. Comparisons between groups at different times were performed using one-way ANOVA and Fisher's tests. Significance was accepted at  $p$  less than 0.05.

## RESULTS

Three weeks after the  $3/4$  nephrectomy, a statistically significant increase in serum urea ( $71.00 \pm 2.66$  vs  $41.00 \pm 0.68$  mg/dL,  $p < 0.05$ ) and creatinine ( $0.828 \pm 0.036$  vs  $0.412 \pm 0.019$  mg/dL,  $p < 0.05$ ) concentrations were found. This increase in renal function markers remained high along the 12 weeks of experimental procedure (Fig. 1).

Comparing with controls, rhEPO treated rats showed a statistically significant progressive in-

crease in Hb, Ht, RBC count, MCV, MCH and RDW, showing at 12 weeks an inverse change, though still presenting significantly higher values; a decrease in platelet count, during the first 9 weeks of rhEPO therapy. When comparing haemorheological data from non-treated CRF and controls, we found only a trend to increased MCV and MCH values and a decrease in percentage of reticulocyte count. Comparing the two groups of CRF rats, we found that rhEPO treated rats presented significantly higher values in RBC count, Hb, Ht and RDW. In both groups of CRF rats, at five weeks, there was a decrease in their values, showing at the end a significantly lower value when compared to controls. No consistent alterations were found in white blood cells in CRF rats, with or without rhEPO therapy.

## CONCLUSION

Partial nephrectomy seems to be a suitable methodology to induce CRF in rats and to study erythropoiesis biology. The rhEPO therapy is associated with an increased erythropoietic stimulation (increase Hb, Ht, RBC count and RDW) and a decrease in platelet count. This method might be useful used to study the cellular and molecular underlying EPO resistance.

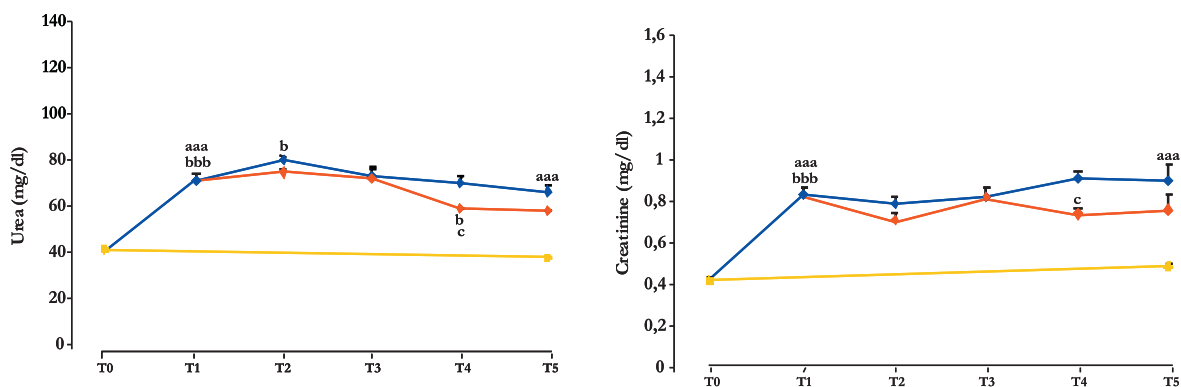
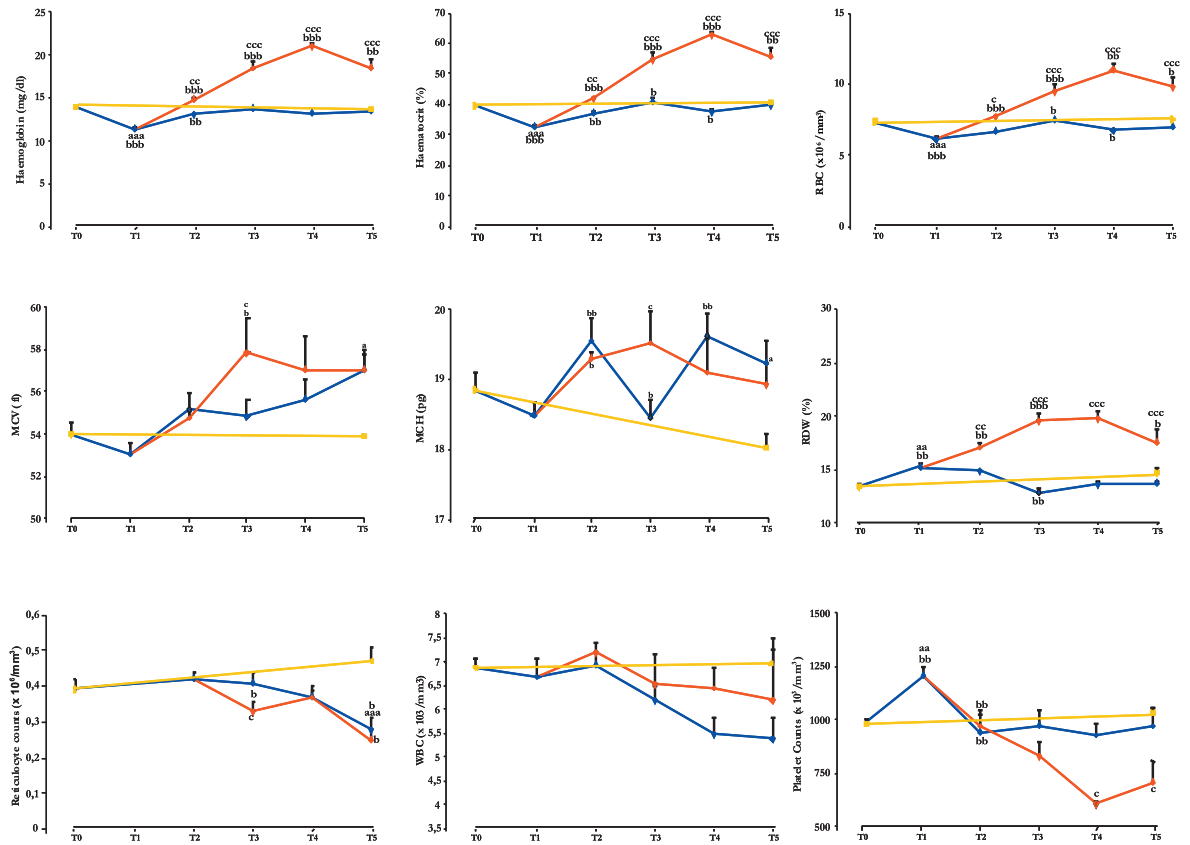


Fig. 1 – Renal function markers (urea and creatinine serum levels) along the 12 weeks of experimental procedure.

—●— Controls; —◆— Non-treated CRF rats; —▲— rhEPO treated

a:  $p < 0.05$ , aa:  $p < 0.01$ , aaa:  $p < 0.001$ , non-treated CRF rats vs controls; b:  $p < 0.05$ , bb:  $p < 0.01$ , bbb:  $p < 0.001$ , T1 vs T0; T2 vs T1, etc.; c:  $p < 0.05$ , cc:  $p < 0.01$ , ccc:  $p < 0.001$ , non-treated CRF rats vs rhEPO treated rats.



**Fig. 2** – Haemorrhological changes during recombinant human erythropoietin therapy along the 12 weeks of experimental procedure.

— Controls; — Non-treated CRF rats; — rhEPO treated

**a:**  $p < 0.05$ , **aa:**  $p < 0.01$ , **aaa:**  $p < 0.001$ , non-treated CRF rats vs controls; **b:**  $p < 0.05$ , **bb:**  $p < 0.01$ , **bbb:**  $p < 0.001$ , T1 vs T0; T2 vs T1, etc.; **c:**  $p < 0.05$ , **cc:**  $p < 0.01$ , **ccc:**  $p < 0.001$ , non-treated CRF rats vs rhEPO treated rats.

## ACKNOWLEDGEMENTS

We are very grateful to Roche Pharmaceuticals to provide the rhEPO used in this work. This study was supported by a PhD grant (SFRH/BD/27688/2006) attributed to E. Costa by FCT and FSE.

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