RESUMOS APRESENTADOS A CONGRESSO

ERYTHROCYTE MEMBRANE BINDING FIBRINOGEN[1]

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High fibrinogen plasma concentration is considered an independent risk factor for cardiovascular diseases. A positive association between plasma levels of fibrinogen and erythrocyte aggregation tendency has been verified in cardiovascular and metabolic diseases as well as in inflammatory situations.

Studies conducted *in vitro* show that soluble and immobilized forms of fibrinogen favour, by a non-specific mechanism, red blood cells (RBCS) aggregates formation.

The aim of the present communication is to present the effects of fibrinogen on RBCs hemorheological properties, nitric oxide mobilization and the identity of the molecular binding target.

When at different shear stress the erythrocyte deformability (ED) was measured, fibrinogen decreases RBCs ability to deform at high shear stress in dependence of erythrocyte membrane band 3 phosphorylation degree. Otherwise fibrinogen promotes the ED in presence of N-ethylmaleimide.

As RBCs are scavenger for nitric oxide that promoting its efflux at microcirculatory network we have, *in vitro*, verified that fibrinogen abrogate its efflux promoting nitrite, nitrate and S-nitrosoglutatione formation.

The presence of the integrin-associated protein (IAP) or CD47 was identified by us, *in vitro*, as the RBCs membrane molecular target which binds fibrinogen in blood samples taken from healthy humans. When RBCs are separated, *in vitro*, by age, according its life span in blood, we verified a greater binding between fibrinogen and younger RBCs in relation to the interaction with the oldest.

The RBCs signal transduction mechanism mediate by fibrinogen has in course which could brings us novel therapeutically targets.

[1] Comunicação apresentada em 9th WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa Portugal

INTERACTION STUDY AT MOLECULAR LEVEL OF THE HEMORHEOLOGICAL PARAMETERS FIBRINOGEN AND ERYTHROCYTE[1]

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The bridging model explains the RBC aggregation mediated by adsorbed macromolecules on adjacent RBC surfaces beyond the electrostatic repulsion forces, membrane strain and mechanical shearing.

Besides a huge data about the effect of fibrinogen on erythrocyte aggregation tendency no binding site has been identified as responsible by the erythrocyte membrane fibrinogen interaction.

The aim of this study was to study the interaction between the erythrocytes membrane with different ages, and plasma fibrinogen in presence and absence of anti-CD47.

Human erythrocytes from healthy donors were separated in a percoll discontinuous gradient. Whole population, old, and young RBC were labeled with: human fibrinogen (Alexa488 or 647) and anti-CD47 (PE). Flow cytometry analyses were made at a BD FACS Calibur analyser. RBC fluorescence images were taken in a confocal microscope Zeiss LSM 510Meta and were analyzed in computer program ImageJ.

Our results show the existence of a specific low binding mechanism between RBC membrane and fibrinogen, being higher in younger than in older RBC. We have observed that the presence of the CD47 antibody diminish the interaction of fibrinogen with the membranes of whole population, young and old RBC. These interactions of was visualized by confocal microscopy.

With this study, for the first time, different level of interaction between RBC, with different ages, and fibrinogen has been verified. Younger RBC establishes a higher interaction with fibrinogen than the older ones, even when erythrocyte aggregation indexes are higher in the later. Furthermore, we have observed a decrease in the fibrinogen interaction with all the three studied populations in the presence of anti-CD47 .This data suggests that CD47 could be a possible target for fibrinogen, or at least it may have a role in the interaction of these acute phase protein with red cell membrane.*(Supported by Fundação para a Ciência e Tecnologia: PTDC/SAU-OSM/73449/2006).*

[1] Poster apresentado em 9th WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa Portugal

NON-NEURONAL CHOLINERGIC SYSTEM IN HUMAN ERYTHROCYTES: BIOLOGICAL ROLE AND CLINICAL RELEVANCE[1]

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Acetylcholine is well-known in the medical setting as one of the most exemplary neurotransmitters. Its ubiquity in nature otherwise suggests a theoretically diverse spectrum of action and an extremely early appearance in the evolutionary process. In humans, acetylcholine and its synthesizing enzyme, choline acetyltransferase, have been found in various non-neural tissues such as the epithelium, mesothelium and endothelium, the muscle, immune cells and blood cells. The widespread expression of non-neuronal acetylcholine is accompanied by the ubiquitous presence of acetylcholinesterase and nicotinic/muscarinic receptors. Structural and functional dissimilarities are evident between the non-neuronal and neuronal cholinergic systems. An increasing body of evidence throughout the last few years has placed acetylcholine as a major cellular signalling molecule in many pathways. Furthermore, numerous erythrocyte physiological events in microcirculation are strongly regulated by acetylcholine. It is then time to revise the role of acetylcholine in humans. Its biological and pathobiological roles must be evaluated in more detail to eventually achieve novel therapeutical targets. Recent evidence from our Unit has noted significant findings about the non-neuronal acetylcholine in red blood cells, with special regard to (i) the red cell rheology, (ii) plasma ions concentrations (iii) nitric oxide (NO) intracellular translocation and metabolism, and (iv) band 3 protein phosphorylation. Significant correlations among nitrosylated molecules, redox thiol status, NO efflux and hemorheological profile have been explored in human erythrocytes.

[1] Comunicação apresentada no 9TH WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal

FIBRINOGEN-DEPENDENT SIGNALLING IN MICROVASCULAR ERYTHROCYTE FUNCTION – IMPLICATIONS ON NITRIC OXIDE EFFLUX[1]

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Experimental evidence has shown that plasma fibrinogen plays a key role as a major cardiovascular risk factor, acting directly to trigger erythrocyte aggregation in occlusive vascular disease. However, due to the complex and hitherto unclear interaction between fibrinogen and the erythrocyte membrane, no study has yet evaluated the effects of fibrinogen, under and above physiological range values, on the erythrocyte nitric oxide (NO) mobilization. Taking into consideration the potential NO-derived molecules, we have raised the hypothesis that fibrinogen, under physiological conditions, may act to influence blood flow via erythrocyte NO modulation. In this *in-vitro* study, whole blood samples were harvested from healthy subjects and erythrocyte suspensions were incubated in the absence (control aliquots) and presence of different fibrinogen concentrations and the levels of NO, nitrite, nitrate and S-nitroglutathione (GSNO) were determined. Our results show, after comparing with control aliquot, that the presence of fibrinogen modulate the NO mobilization in erythrocyte by: (1) decreasing the erythrocyte NO efflux levels ($p<0.001$); (2) increasing the levels of intraerythrocytic NO oxidative metabolites namely nitrite ($p<0.0001$) and nitrate ($p<0.0001$); (3) enhancing the formation of GSNO ($p<0.001$). In conclusion, this study gains new insights into an unknown mechanism by which fibrinogen modulates the erythrocyte capacity to supply nitric oxide, which effects on inflammation profiles (generally associated with blood hyperviscosity and hyperaggregation) still need to be elucidated. Also, increased erythrocyte GSNO levels may be associated with platelet NO metabolism, its activation status and hypotension, which may be extremely relevant in the clinical setting as biomarkers.

[1] Poster apresentado em 9TH WORLD CONGRESS ON MICROCIRCULATION. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa Portugal

FIBRINOGEN BINDING TO ERYTHROCYTE MEMBRANE: CD47 A POSSIBLE MOLECULAR TARGET[1]

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During the last decades, several studies have shown that high levels of plasma fibrinogen induce erythrocyte hyperaggregation, suggesting a possible interaction between this protein and red blood cells, (RBCs). Importantly an increased of the thromboembolic risk, in several cardiovascular disorders, is usually associated to both of these hemorheological parameters. There is a theory saying that the fibrinogen interacts with the surface of RBCs due to specific and non-specific mechanisms could be the main trigger to RBC hyperaggregation under inflammatory conditions. We hypothesize that human RBCs are able to specific bind soluble fibrinogen, and a membrane molecular target should be responsible for this phenomenon, although no one until now has pointed out one.

In this work we have applied two different techniques: fluorescent confocal microscopy and flow cytometry. RBCs were isolated and separated in different age fractions from whole blood collected from healthy donors. The data collected in both techniques support the idea that soluble fibrinogen binds to human RBC membrane interacting with it in an age-dependent manner. The youngest RBCs have shown to have higher interaction with soluble fibrinogen than the oldest. Importantly in this work we also point out to a specific molecular target for soluble fibrinogen at RBC membrane, the integrin-associated protein (IAP) or CD47.

Our work describes for the first time a specific and age-dependent interaction of soluble fibrinogen with human RBC membrane and we point out CD47 (human RBC isoform) as a possible molecular target for this acute phase protein. This interaction may well be responsible for a specific mechanism that under inflammatory conditions triggers erythrocyte hyperaggregation.

[1] Comunicação apresentada em XXI INTERNATIONAL FIBRINOGEN WORKSHOP. Estudo realizado na Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular, Instituto de Bioquímica, Faculdade de Medicina, Universidade de Lisboa)