COMUNICAÇÕES A CONGRESSOS / CONGRESS COMMUNICATIONS

RESUMO DE COMUNICAÇÃO APRESENTADA À *25TH CONFERENCE OF THE EUROPEAN SOCIETY FOR MICROCIRCULATION, INTEGRATING VASCULAR BIOLOGY AND MEDICINE, 26-29 AGOSTO 2008, BUDAPEST, HUNGARY.*

ANALYSIS OF FIBRINOGEN BINDING TO THE ERYTHROCYTE MEMBRANE BY FLOW CYTOMETRY

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Fibrinogen is an acute phase protein that participates in microvascular events in hemostasis, inflammation and hemorheology. Regarding this latter, plasma fibrinogen is associated with erythrocyte hyperaggregation. The interaction of fibrinogen with the erythrocyte membrane is not fully understood, with published data supporting both specific and non-specific binding mechanisms.

The objective of our study was to develop a flow cytometry methodology to analyze the interaction of fibrinogen with the erythrocyte membrane.

Human blood was used, and independent flow cytometry assays were performed with either whole blood or isolated erythrocytes. In both cases the erythrocytes were identified based on the forward and side scatter characteristics, and further checked using the erythrocyte membrane marker glycophorin A. The samples were incubated with different concentrations of AlexaFluor488-labeled fibrinogen for different time points. Our results show that flow cytometry reliably identifies binding of the fluorescent fibrinogen to the erythrocyte.

We are using this approach to help characterize the potential erythrocyte membrane binding site for fibrinogen. More information about this possible fibrinogen-erythrocyte binding site could lead new therapeutically approaches to address the erythrocyte hyperaggregation responsible for a significant increase of the risk of cardiovascular disorders.

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