S29: Role of gasotransmitters (NO, CO and H2S) in blood cell functions and the molecular mechanisms of their microrheology alterations

S25-1 Leukocytes as a link between inflammation and erythrocyte nitric oxide

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Vascular endothelial cells change its phenotypes to participate in the acute inflammation, which involve a fast and a slower response in close relation with the white blood cells approaching them. Also, release of chemical signals like histamine, prostaglandins and nitric oxide (NO) by endothelial cells occurs. NO liberate to vessel lumen is scavenged by erythrocytes through its membrane band 3 protein. The availability of erythrocytes in deliver or scavenger NO is in accordance with the phase of inflammation. For instance in non-survivor sepsis`s patients the amount of NO efflux was higher, than on survivor ones. The homeostatic human body mechanisms try, through NO liberated by erythrocytes, to compensate the vasoconstriction observed by the unequal blood flow and decrease microvascular blood flow index, using sub-lingual microcirculation. The intravital microscopy coupled with fluorescence detectors and confocal microscopy allows us to visualize, in vivo, the acute inflammation development. Specific software treated the video-images captured in order to obtain hemodynamic data, leukocyte rolling velocity, number of leukocytes rolling and adherent in to endothelial wall. The efflux of NO from erythrocytes decreases during the acute phase of inflammation as verified in an animal model of acute inflammation with labeled neutrophils, where increased number of neutrophil rolling and adhesion was observed. The erythrocyte deformability decrease observed favoring the increase of the whole blood viscosity and consequently pouching the neutrophil to endothelium of post capillary venules.

S29-2 Contribution of fibrinogen to erythrocyte scavenger nitric oxide

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Fibrinogen (Fib) is a plasma protein participant in the hemostatic and the hemorheological process known also as one of the acute phase inflammation factors when at high concentrations. Nitric oxide (NO) liberated by endothelium cells or lymphocytes enters into erythrocytes and binds to hemoglobin and to glutathione originating reservoirs of NO molecules. Fib binding to CD47 erythrocyte membrane protein decreases nitric oxide (NO) efflux from erythrocyte and increases the NO derivatives (NOx) molecules. Mimicking an hyperfibrinogenemia condition NO efflux and NOx levels change according a signal transduction mechanism under dependence of cAMP concentration and erythrocyte membrane band 3 protein, protein kinase C, phosphodiesterase -3 , phosphorylation status and also adenylyl cyclase enzyme activity and acetylcholinesterase enzyme conformation and active state degree. Some of the above biomolecules are already therapeutic targets which the mechanism of action is used in glaucoma and obesity can be explained by the erythrocyte availability in NO.

S29-3 Role of nitrogen oxide and hydrogen sulfide as signaling molecules in the change of the red blood cell microrheology in patients with type 2 diabetes mellitus

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Introduction. It is known that type 2 diabetes mellitus (DM-2T) is associated with impaired blood rheology and red blood cells (RBCs) too. There are some reports that RBC microrheology (RBCM) varies under the influence of nitric oxide (NO).

The aim of the study was to estimate the effect of gasotransmitters (GT) donor: sodium nitroprusside (SNP) and sodium hydrosulfide (NaHS - hydrogen sulphide donor) on RBCM in normal conditions and under DM-2T.

Methods. Erythrocytes were incubated with: 1) SNP (100 μM); 2) NaHS (100 μM); 3) methylene blue (MB, 20 μM) and 4) glibenclamide (GkL, 10 μM). The RBC deformability (RBCD) and their aggregation (RBCA) after incubation with each of the GT donor was recorded.

Results. Incubation of cells with the SNP has led to an increase in RBCD by 12% ($p < 0.01$), and RBCA was decreased by 34% ($p < 0.01$). The H_2S donor had a similar effect on RBCM – RBCD significantly increased by 8%, and RBCA decreased by 17% ($p < 0.05$). MB - inhibitor of soluble guanylate cyclase (s-GC) reduced the positive effect of SNP on red cell microrheology. Blocking K^+_{ATP} channels with GkL did not eliminate the positive effect of NaHS on RBCM. It was found that after combined action of "GkL + NaHS" RBCD was higher than in the control ($p < 0.01$), and RBCA by 30% less ($p < 0.01$).

Conclusion. Taken together, GT donors have a positive effect on red cell microrheology. The molecular target for NO in erythrocytes is p-GC, which was confirmed in experiments with its inhibition, whereas for the role of K^+_{ATP} channels, as a target for hydrogen sulphide, under these conditions was not revealed.

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S29-4 Change of microrheological characteristics of erythrocytes under the influence of donors of gasotransmitters NO and H_2S: in vitro study

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Background. The signal role in cellular reactions of gaseous transmitters (NO and H2S) is known. However there are only a few studies on the effect of NO on red blood cell aggregation and deformability (RBCD). Role of H2S as a signaling molecule in the RBC microrheology changes remains unexplored. The aim of this study was to investigate the effect of NO and H2S donors on microrheological parameters of RBCs.

Methods. RBC microrheology was recorded after cell incubation with: 1) NO donors – spermine NONOate (10–5 M) and sodium nitroprusside (SNP, 10-4 M); 2) hydrogen sulphide donor – (NaHS, 10-4 M); 3) a blocker of K+(ATP) channels – glibenclamide (10-5 M). RBC suspension prepared in drug-free solution was used as a control sample.

Results. RBCs under the influence of both types of the gasotransmitter (GT) donors was reduced by an average of 25% ($p < 0.01$). Under these conditions, a moderate increase of RBCD (by 7-14%, $p<0.01$) was also found. We incubated RBCs with glibenclamide, a blocker of K+(ATP) channels and observed a decrease in aggregation of 27% ($p<0.01$) and an increase in cell deformability by 8% ($p<0.01$). It is important to note that blocking the potassium channels with glibenclamide did not eliminate the noticeable microrheological effect of the hydrogen sulphide donor (NaHS).

Conclusion. Obtained data allow us to conclude that both types of gasotransmitters may positively affect RBC microrheology: significantly reduce their aggregation and moderately but statistically significant increase the RBCD. It is likely that with changes in RBC microrheology, both gasotransmitters acted on the same key intracellular molecular target. This is probably gaunilate cyclase.

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