ERYTHROCYTE COMPONENTS CONTRIBUTE TO NITRIC OXIDE AVAILABILITY AT SITES OF INTRAMURAL MICROHAEMORRHAGE: A POSSIBLE MECHANISM OF VASCULAR CALCIFICATION AND ATHEROGENESIS

M. Pavlaki, K. Moiko, A.Thomaidis, A. Kourkoulis, A. Markakis, G.Chalikias, S. Konstantinides, D. Tziakas

BACKGROUND: Recent experimental work by our group dissected the role of red blood cells (RBCs) in atherosclerosis, atherothrombosis and vascular calcification, both *in vitro* and *in vivo*. Specifically, we demonstrated nitric oxide (NO)-dependent enhancement of vascular smooth muscle cell osteogenesis. NO originated from RBC NO synthase (eNOS) located in the cytoplasmic part of RBC membranes. RBC membranes exerted osteoinductive effects, while intact RBCs did not. The aim of the present work was to investigate the pathomechanisms underlying the differential osteoinductive potential of RBC membranes as opposed to intact or ruptured RBCs. We focused on factors modulating NO production, such as arginase activity, and NO degradation after its synthesis, including haemoglobin-dependent NO scavenging.

MATERIALS AND METHODS: RBCs were isolated from human blood. Intact, lysed RBCs and RBC membranes, were examined *in vitro* for enhancement of osteogenic differentiation and calcification of aortic smooth muscle cells (AoSMC) in culture, visualized by Alizarin staining. In this assay, oxidative neutralisation of cytoplasm or intact RBCs served to evaluate the NO scavenging activity of oxyhaemoglobin Fe^{2+} species. Arginase activity and content were assessed using an inhibitor of the enzyme in the calcification assay and by sample immunoblotting, respectively.

RESULTS: Using the calcification assay of AoSMCs, it was shown that blood from different donors varied with regard to the osteoinductive potential of the RBC membranes. The NO donor DETA-NONOate was used as a reference. The calcification effect of RBC membranes was enhanced by physical contact with the AoSMCs. RBC membranes markedly enhanced calcification, in contrast to RBC lysates or intact erythrocytes which exerted almost no effect or even inhibition of calcification. The osteoinductive effects of RBC cytoplasm or intact RBCs were enhanced following neutralisation of oxyhaemoglobin. A similar enhancing effect was obtained when cytoplasm or intact cells were examined in the presence of the arginase inhibitor L-NORVA. Of note, calcification induced by RBC membranes remained unchanged following the above steps. Arginase was detected in both the RBC cytoplasm and the membranes by western blot analysis. Based on band densitometry, the content of arginase was approximately one order of magnitude higher in the cytoplasm compared to RBC membranes.

CONCLUSIONS: Our results suggest arginase competition with eNOS for their common substrate L-arginine in the RBC cytoplasm compared to RBC membranes. This mechanism may explain, at least in part, the enhanced calcification effect of RBC membranes as opposed to intact or damaged extravasated RBCs. Incomplete clearance of RBCs at sites of intraplaque microhaemorrhage may result in depletion of quenching components, such as cytoplasm haemoglobin or arginase, resulting in a ‘gain of NO function’ in the remaining RBC membranes in the lesion and thus promoting vascular calcification.

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