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Activity of Caspase-3 in Erythrocytes of Patient with Anemic Conditions

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Aim of the study: Iron-deficiency anemia (IDA) and anemia of chronic disease (ACD) are the most frequent form of anemic conditions. The cause of IDA is iron deficiency in organism while ACD develops as concomitant of different pathologies. It is still considered that under these pathologies «defective» erythrocytes are died by extravascular hemolysis. At the same time decrease of erythrocyte's life span can be related with initiation of eryptosis. The tissues hypoxia is one of anemia consequences that in turn cause an oxidative stress. Previously we established that oxidative stress in erythrocytes *in vitro* was accompanied by activation of caspase-3. The aim of this work was to evaluate caspase-3 activity in erythrocytes of patients with IDA and ACD.

Material and methods: The samples of venous blood from healthy human volunteers (HV) were obtained from RSPC of Transfusiology and Medical Biotechnologies, Ministry of Health. Samples of blood from patients with IDA and ACD were obtained from Minsk Consulting and Diagnostic Centre. Erythrocytes were derived from blood by centrifugation and subsequently were washed in 155 mM NaCl (4C, 2000g, 5 min). Cells with exposed phosphatidylserine (PS) well known marker of eryptosis was detected using FITC-annexin V. Activity of caspase-3 was evaluated using CaspGlow^{TM} kit containing specific caspase-3 substrate FITC-DEVE-fmk. Fluorescence measurement was carried out by flow cytometric analysis on BD FACS Canto II (Becton Dickinson, USA). Obtained data expressed as arithmetic means ± SEM of at least 6–12 independent experiments and statistical analysis was made by Mann-Whitney U-test and Wilcoxon signed-rank test. Statistical significance of the data was defined as follows p <0,05 and p < 0,01.

Results: In both erythrocyte populations HV and patients with anemias was shown presence of cells with exposed PS. At the same time average number of such erythrocytes in blood samples of patients with anemias were 7,1±1,5% and 5,2±0,7% for IDA and ACD respectively whereas for HV – 1,9±0,2%. This data indicated about increase of eryptotic cells in blood samples from patients with anemic conditions. However significant differences in fluorescence intensity of FITC-annexin V between PS exposing erythrocytes of HV and patients with anemia were not detected. It was established that in all group under investigation occur presence of cells with activated caspase-3. For example average number of such caspase-positive erythrocytes in blood samples of patients with anemias were 1,3±0,3% and 0,8±0,2% for IDA and ACD respectively whereas for HV – 0,3±0,1%. It should be noted that fluorescence intensity of FITC-DEVE-fmk substrate had the same values in caspase-positive cells for both HV and patients with anemia.Obtained results suggested that in erythrocyte's population from patient with anemic conditions (IDA and ACD) tend to increase number of eryptotic cells developed by caspase-dependent pathway.

Keywords: Anemia, erythrocytes, phosphatidylserine, caspase-3.