MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Hydrogen Peroxide on Ejection of Cell Nucleus from Pigeon Erythrocytes and State of Membrane Lipids

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The nuclei are ejected from the pigeon erythrocytes and apoptotic vesicles form in these cells in the presence of hydrogen peroxide. Hydrogen peroxide intensifies LPO processes and changes phospholipid content. The relative content of phosphatidylcholine, sphingomyelin, and phosphatidylserine decreased, while that of phosphatidylethanolamine and lisophosphatidylcholine increased. The content of unsaturated fatty acids also decreased under these conditions. Presumably, these changes in the lipid phase of the erythrocyte membrane are a mechanism preparing the cell to nucleus ejection and apoptosis.

Key Words: *apoptosis; hydrogen peroxide; phospholipids; lipid peroxidation; fatty acids*

Apoptosis can be triggered by numerous external and internal signals. This process is aimed at removal of old cells and cells with impaired differentiation and damaged genetic matter from the organism [3]. Apoptosis is a pathway of blood cell renewal. Despite universal nature of apoptosis, the mechanisms of its triggering differ in the organisms at different levels of evolution. Irrespective of the nature and sources of external signals triggering apoptosis directly or through inductors, their effects on the cell cannot be realized without interactions with cell membrane and its components, including phospholipids. Apoptosis can be realized primarily by impairing the membrane permeability and modification of its physicochemical characteristics [9]. However, the data on the role and involvement of lipids in apoptosis are scanty. Apoptosis inductors, including H_2O_2, are often used in studies of apoptosis mechanisms [7].

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We studied morphological and biochemical changes in the pigeon erythrocytes in the presence of H_2O_2.

MATERIALS AND METHODS

Pigeon blood and erythrocytes were analyzed. Apoptosis inductor H_2O_2 in different concentrations was *in vitro* added to pigeon blood [7]. Blood stabilized with heparin was incubated with KCN (0.5 mM) for inhibiting catalase and peroxidase activities. Morphological characteristics of erythrocytes were evaluated using Lyumam P8 microscope with computer-aided data recording. For studies of biochemical changes, erythrocytes were separated by centrifugation, washed in normal saline; erythrocyte lipids were extracted as described previously [5]. Fatty acid methylation was carried out [8]. LPO products, conjugated dienes, and MDA were evaluated by the method described previously [4]. In order to evaluate qualitative and quantitative distribution of phospholipids, lipids were fractionated by thin layer chromatography on 6×6-cm plates in a

system of solvents [6]. Phospholipid fractions were identified using R_f values and specifically staining reagents [2]. The content of individual phospholipid fractions was evaluated by inorganic phosphorus [10]. In order to evaluate changes in fatty acid composition of individual fractions, phospholipids were separated on 20×10 cm plates and eluted in a chloroform:methanol (2:1) system. Fatty acids were analyzed on a Chrom-4 gas-liquid chromatograph with programmed temperature (from 170 to 225°C) [4]. The results were processed using Microsoft Excel 2000 electronic tables IBM IP2 and Stat2 software.

RESULTS

Changes in erythrocyte morphology depended on H_2O_2 concentration and duration of exposure. Incubation of blood at 10-40°C for 24 h in the presence of 1 mM H_2O_2 caused no changes in erythrocyte morphology. Increasing H_2O_2 concentration to 20 mM led to modification of their structure and composition. Erythrocytes without nuclei (Fig. 1) were seen after 6-h incubation at 25°C. It is known that mature mammalian erythrocytes have no nuclei, while avian cells have nuclei. The mechanism of nucleus ejection in these organisms, particularly in birds, remains little studied. Nucleus ejection in pigeon erythrocytes resembled "budding", that is, similarly as in mammalian cells, the cell gave a sprout containing the nucleus. The initial cell membrane was then restored. Erythrocytes without nuclei were somewhat smaller than full-value cells. Longer exposure to H_2O_2 and higher temperature increased the number of nucleus-free cells and led to the for-

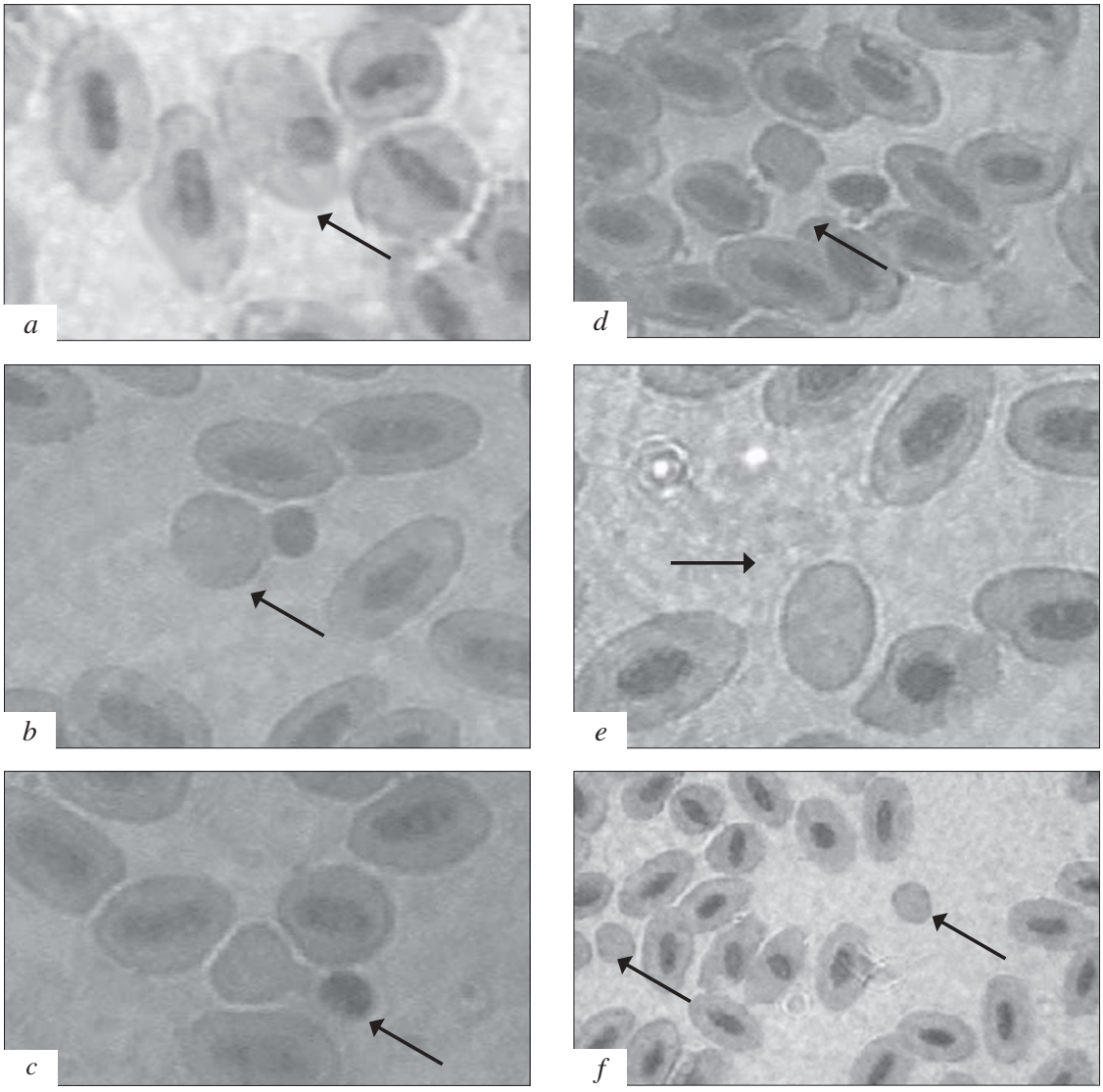

Fig. 1. Nucleus ejection from pigeon erythrocyte induced by H_2O_2. *a*, *b*, *c*) erythrocytes at the stage of nucleus ejection (arrows); *d*, *e*) nucleus-free erythrocytes (arrows); *f*) apoptotic vesicles (arrows; 3.8% of total counts of erythrocytes after 24-h incubation at 40°C).

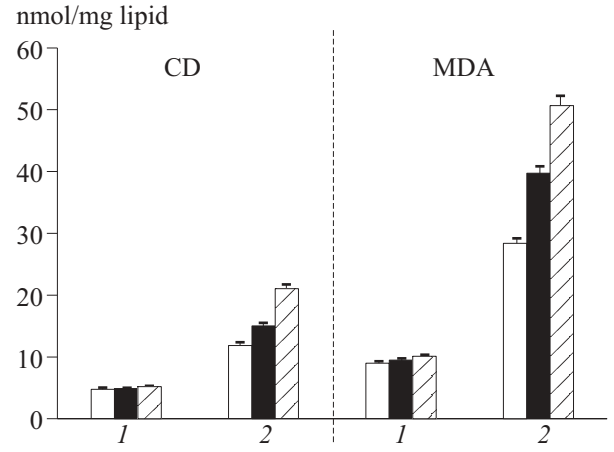

Fig. 2. Content of conjugated dienes (CD) and MDA in pigeon erythrocytes after exposure to H_2O_2. Light bars: no incubation; dark bars: 12-h incubation; cross-hatched bars: 24-h incubation. *1*) control; *2*) H_2O_2.

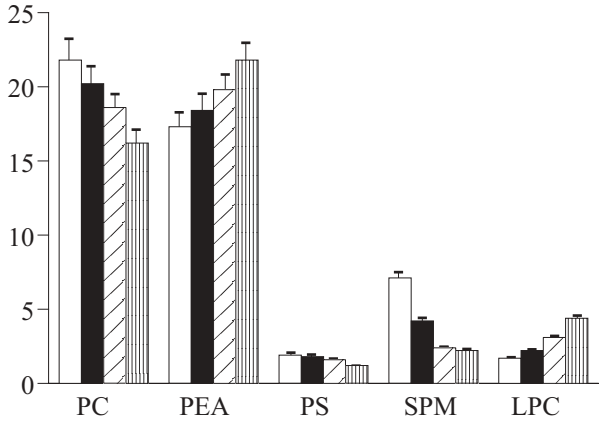

Fig. 3. Effect of H_2O_2 on phospholipid content in pigeon erythrocytes (μg P_i in conversion to 1 mg total lipids P_i). Light bars: control; dark bars: after addition of H_2O_2; cross-hatched bars: 12-h incubation; vertically hatched bars: 24-h incubation. PC: phosphatidylcholine; PEA: phosphatidylethanolamine; PS: phosphatidylserine; SPM: sphingomyelin; LPC: lisophosphatidylcholine.

mation of new bodies. These bodies were closed, spherical, similar to nucleus-free erythrocytes, but smaller. Presumably, they formed as a result of further "budding" of nucleus-free erythrocytes and were apoptotic vesicles.

Hence, H_2O_2 induces nucleus ejection from pigeon erythrocytes and formation of apoptotic vesicles.

Incubation of erythrocytes with H_2O_2 was paralleled by an increase in the content of LPO products (Fig. 2). H_2O_2 is a source of ROS initiating LPO processes [1]. Despite appreciable increase in the content of LPO products, the cells mainly retained their structure. Lipid oxidation depends primarily on the degree of fatty acid saturation, qualitative and quantitative distribution of phospholipids. These distributions can compensate the negative effects of LPO on cells. The resistance is determined by cell capacity to triggering these mechanisms. Exposure to H_2O_2 leads to modification of the quantitative ratio of phospholipids without changing their qualitative composition (Fig. 3). The percentage of phosphatidylcholine, sphingomyelin, and phosphatidylserine decreased, while that of phosphatidylethanolamine and lisophosphatidylcholine increased. Decreased content of phosphatidylcholine, sphingomyelin, and phosphatidylserine attests to impaired liquid properties of the membrane. Presumably, these changes in structural and functional state of the membrane are aimed at the maintenance of structural integrity and functional activity of erythrocyte membranes.

This hypothesis is confirmed by changes in fatty acid composition of individual phospholipid fractions (Fig. 3). Addition of H_2O_2 caused changes in the quantitative distribution of fatty acids. The type of these changes depended on the duration of exposure and morphological status of erythrocytes. H_2O_2 increased the percentage of saturated fatty acids and decreased the percentage of unsaturated ones in all phospholipid fractions, which increased the unsaturation coefficient. The decrease in the percentage of unsaturated fatty acids in all phospholipids was mainly at the expense of oleic and linoleic acids. These shifts were more pronounced for phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin, less so for lisophosphatidylcholine. After 24-h incubation their percentage decreased 1.4- and 4-fold in phosphatidylcholine, 1.4- and 2.4-fold, respectively, in phosphatidylserine. After the same exposure the fatty acid composition of phosphatidylethanolamine contained no oleic acid and there was no linoleic acid in sphingomyelin. The increase in the content of saturated fatty acids was largely at the expense of palmitic and stearic acids, except the phosphatidylethanolamine fraction (main shifts in the levels of myristic and behenic acids). These data confirm that H_2O_2 causes deep changes in membrane structure and function, primarily due to changes in the membrane lipid bilayer. Increased content of LPO products and decreased fatty acid saturation in erythrocyte membranes increases the possibility of cell destruction by oxidative processes.

Hence, H_2O_2 induces ejection of the nuclei from erythrocytes with the formation of apoptotic vesicles and this is paralleled by changes in the membrane lipid composition. Changes in the quantitative and qualitative ratio of lipids and LPO products can be a mechanism triggering apoptotic processes and their prevention.

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REFERENCES

1. A. V. Kaluev, *Biokhimiya*, **63**, No. 9, 1305-1306 (1998).
2. M. Kates, *Lipidology Techniques* [in Russian], Moscow (1975).
3. V. D. Samuilov, A. V. Oleskin, and E. M. Lagunova, *Biokhimiya*, **65**, No. 8, 1029-1046 (2000).
4. I. D. Stal'naya and T. G. Garishvili, *Modern Methods in Biochemistry* [in Russian] (1977), pp. 63-68.
5. E. G. Blight and W. I. Dayer, *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).
6. R. M. Broekhuyse, *Biochim. Biophys. Acta*, **260**, 449-459 (1968).
7. T. Jabs, *Biochem. Pharmacol.*, **57**, 231-245 (1999).
8. W. R. Morrison and L. M. Smith, *J. Lipid Res.*, **53**, 600-608 (1964).
9. M. M. Taketo and M. Sonoshita, *Biochim. Biophys. Acta*, **1585**, No. 2-3, 72-76 (2002).
10. V. E. Vaskovsky, E. Y. Kostevsky, and J. Vasendin, *J. Chromatogr.*, **144**, 129-141 (1975).