THE EFFECT OF POLY(ADP-RIBOSE) POLYMERASE INHIBITOR 4-HYDROXYQUINAZOLINE ON OOGENESIS AND CELL DEATH PATHWAYS UNDER EXPERIMENTAL IMMUNE COMPLEX-MEDIATED INJURY IN MICE

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Introduction. Recently, the nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1) has been actively studied. In the face of moderate genotoxic stress, PARP-1 promotes DNA repair and maintains genome stability [1, 2, 3]. With excessive activation, the pathogenetic role of PARP-1 (involvement in the pathogenesis of a number of diseases, including autoimmune ones) is manifested [4, 5]. It is known that in the pathogenesis of many reproductive system organ diseases, including the ovaries, immuno-inflammatory processes play an essential role. However, the role of PARP-1 in inflammatory diseases of the female reproductive system has not been practically studied. At the same time it was shown the important physiological role of the enzyme in the reproductive function, including in gametogenesis [6, 7, 8]. PARP-1 is expressed in oocytes and follicular cells [7, 8]. Studies in experimental models of several diseases, including autoimmune (ones), showed a pronounced protective effect of PARP inhibitors [4, 9, 10, 11]. These data served as the basis for the pharmacological PARP inhibition in various inflammatory diseases. In this study, we used a murine model of immune complex-mediated pathology induced by immunization with bovine serum albumin (BSA) to investigate the effect of PARP-1 inhibitor 4-hydroxyquinazoline (4-HQ) on oogenesis and cell death pathways.

Material and Methods. The experiments were carried out on mature female mice (18–20 g, inbred strain CBA). The mice (n=17) were immunized with BSA intravenously 6 times every 7 days according to the scheme: 1) 150; 2) 175; 3) 200; 4) 225; 5) 250 and 6) 275 mg of BSA per kg of body weight. Seven days after the last injection, the mice were euthanized under ether anesthesia and their ovaries were sampled. The control mice received equivalent volumes of normal saline. The nine mice immunized with BSA were injected with 4-HQ («Sigma», USA) intraperitoneally (100 mg/kg of body weight) twice each week.

Results. We have found that administration of BSA resulted in immune cells activation, elevated level of immune complexes in serum and their enhanced deposition in the liver, spleen, aorta and kidney as well as resulted in vascular and parenchymal damage of these organs. Immunization increased the number of necrotic lymph node cells (by 4.5 times) and necrotic thymocytes (by 3.5 times). The percentage of apoptotic immune cells did not change significantly. PARP inhibition exerted a strong cytoprotective effect: viability of thymus and lymph node cells was increased mainly due to reduced level of necrosis. As shown by comet assay, DNA damage index of immune cells was increased 4,0 times in mice with immune complex-mediated pathology, $P<0.001$. The percentage of thymic cells with strong DNA damage was increased to 77% under immunization (compared to 1.5% in control mice) and the percentage of such cells from lymph nodes was increased to 80% (compared to 0% in control), in both cases $P<0.001$. Genotoxic stress was reduced by treatment of immunized mice with 4-HQ: the percentage of lymphocytes with strong DNA damage was significantly decreased. Immunization caused ovary dysfunction in female mice. The administration of BSA induced a significant reduction in granulosa

cell viability mainly due to enhanced necrotic cell death that was accompanied by impairment of the meiotic maturation of oocytes: the number of oocytes at metaphase I and metaphase II decreased significantly compared to that of control mice (corresponding data are 72.59±3.4% and 32.91±3.6% vs. 88.64±2.7% and 48.39±3.0% in control group; P<0.05). The treatment of the immunized mice with 4-HQ improved granulosa cell viability: diminished necrosis, while the percentage of apoptotic cells remained unchanged. Inhibition of PARP also improved the meiotic maturation of oocytes at metaphase I (86.44±2.5%; P<0.05) and II (45.11±3.9%; P<0.05) in comparison to immunized mice.

Conclusion. Therefore we demonstrate that PARP-1 activation may be involved in the pathogenesis of the experimental immune system failure. PARP inhibition exerted the protective effect that may be mediated, at least partially, through the attenuation of necrosis. Thus our results give evidence that inhibition of this enzyme may constitute a perspective target in immune complex diseases prevention and therapy.

Keywords: immune-complex injury, immune cells, oocytes, follicular cells, PARP inhibition, mice.

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BIOCHEMISTRY OF THE VITREOUS BY THE HUMAN EYE IN NORMAL AND PATHOLOGICAL CONDITIONS

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