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Original Research Article

Inhibition of Poly (ADP-Ribose) Polymerase (PARP) Protects against Experimental Immune Complex-Induced Ovarian Failure in Mice

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ABSTRACT

The effects of PARP inhibitor, 4-hydroxyquinazoline (4-HQ), on ovarian damage were studied in mouse model of immune complex (IC) injury induced by immunization with bovine serum albumin (BSA). The BSA injections impaired the meiotic maturation of oocytes, enhanced necrosis and reduced expression of COX2, GREM1 and HAS2 genes in follicular cells. Treatment of mice with 4-HQ exerted beneficial effect on the ovarian function and oocyte quality in IC–mediated injury: improved oocyte maturation, diminished necrosis and significantly enhanced the expression of mRNA for COX2 and GREM1 in follicular cells.

Keywords: Oocytes, Follicular cells, Immune complex diseases, PARP inhibitors, Mice.

INTRODUCTION

Female fertility is affected by a variety of environmental and genetic factors that can alter folliculogenesis at various levels. These factors include a wide spectrum of endogenous genotoxic agents produced during immune-mediated inflammation that may lead to DNA damage and cell death. DNA strand breaks activate enzymatic activity of nuclear enzyme PARP1 (up to 500-fold), which participates in DNA repair and genomic integrity. The enzyme uses NAD^+ as substrate to form large negatively charged polymer of poly (ADP- ribose)-(PAR) and attaches them to acceptor proteins, including his tones, DNA repair proteins, transcription factors. [1] This post-translational modification modulates protein function and, in addition to its classical role as a DNA repair mediator, PARP1 is also implicated in the regulation of a wide range of important cellular processes including transcriptional regulation, chromatin modification, cell homeostasis, proliferation, and death. [2,3] As for ovary, there is evidence that PARP1 is involved in oogenesis, folliculogenesis and atresia. [4,5] Thus, optimal PARP1 expression and activity are essential for variety of cellular processes, but excessive activation of the enzyme has been shown to contribute to tissue injury and inflammatory disorders. PARP1 participates in the pathogenesis of various immune-mediated diseases (rheumatoid arthritis, autoimmune nephritis, atherosclerosis, autoimmune multiple sclerosis etc) mainly through the activation of proinflammatory transcription factors (such as NF-κB, AP-1) and the increase in a necrotic type of cell death. [2,3] It was shown in the pre-clinical models that many acute and chronic inflammatory processes, having different aetiopathogenesis and occurring in different organs or being systemic, can be attenuated by PARP inhibition. [1,3,6,7]

A variety of immune-mediated inflammatory disorders are associated with

formation and tissue deposition of immune complexes (ICs), but the role of PARP in the development of immune complex (IC) induced injury is poorly studied. Since, as we showed earlier, ICs can induce reproductive function disturbances, [8,9] it was of interest to study the influence of PARP inhibition on ovarian function in this pathology. We investigated the effects of 4-hydroxyquinazoline (4-HQ), a potent PARP1 inhibitor, on the oocyte meiotic maturation, as well as on apoptotic and necrotic death of follicular cells in the mouse model of IC disease induced by immunization with bovine serum albumin (BSA). In addition we detected the gene expression (hyaluronic acid synthase 2 - HAS2, cyclooxygenase 2 - COX2 and gremlin 1 - GREM1) in cumulus cells under these experimental conditions.

MATERIALS AND METHODS

The experiments were carried out on mature female mice (18-20 g, inbred strain CBA; Experimental Animal Laboratory of Bogomoletz Institute of Physiology). All procedures in this study were approved by the Animal Care Committee of Bogomoletz Institute of Physiology in accordance with the International Principles of the European Convention concerning the protection of vertebrates. The mice were randomly divided into three groups: 1/ control group, n=8; 2/ mice immunized with BSA («Sigma», USA), n=8; 3/ mice immunized with BSA and receiving 4-HQ, n=9.

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 instead of BCA and were treated similarly to those of the experimental group. The nine mice immunized with BSA were injected with 4-HQ («Sigma», USA) intraperitoneally (100 mg/kg of body weight) twice each week.

Follicles were separated from ovaries and then counted. Cumulus-oocyte cellular complexes (COCs) were extracted mechanically and were cultured at 37^{o}C in Dulbecco's modified Eagle's medium (Sigma, USA), supplemented with 5% fetal bovine serum and antibiotics. The number of oocytes with germinal vesicle breakdown (metaphase I) was counted after four hours, while the number of oocytes forming the first polar body (metaphase II) was estimated by light microscopy after 20 hours of culture.

Follicular cell isolation and the estimation of viable, apoptotic and necrotic cell numbers (by their double vital staining with fluorescent dyes Hoechst 33342 and propidium iodide) were performed as earlier. [7]

For the immunocytochemical detection of PARP activity, polyclonal rabbit anti- PAR antibody (BD Pharmingen) was used. PAR immunocytochemistry was performed mainly as described. [10] The immunoreactive signal was developed using diaminobenzidine as a substrate ("Sigma", USA). The follicular cell smears were examined by light microscopy (oil immersion, 100 x objectives). PAR immunoreactivity was assessed by semi quantitative scoring which includes (i) the percentage of positive cells (0-100%) and (ii) the signal intensity (5-point scale). The scoring for the staining intensity corresponded to 0, negative; 1, weak staining; 2, moderate positive; 3, strong positive and 4, very strong staining. The semi quantitative immunocytochemical (ICC) score for PAR level was provided by the multiplication of the percentage (0–100%) of positive stained cells by the factor (1-4) corresponding to the staining intensity of the cells.

Reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA for RT-PCR analysis was obtained from cumulus cells using the mRNA extraction kit (Invitrogen, USA). First-strand cDNA of

cumulus cells obtained after reverse transcription was used for carrying out of PCR with application of specific primers for COX2, GREM1, and HAS2 genes and housekeeping gene GAPDH (Table 1). PCR was performed in termocycler Gene Amp System 2700 ("Applied Bio systems", USA). The resulting PCR products were then subjected to electrophoresis on a 2.5 % agarose gel, stained with ethidium bromide and analyzed by UV transilluminator and software ViTran ("Biokom", Russia).

Table 1: List of PCR primers used for experiments and PCR product size

Gene	Sequence of primers (forward, reverse)	Product size/bp
HAS2	forward 5'- CCTCCAGTTAGTGTCTGGGTTC -3' reverse 5'- CTGTGCAGCTATTCCTGTGTTC -3'	409
COX2	forward 5'- GAAGGAACTCAGCACTGCATC -3' reverse 5'- CAGTCCGGGTACAGTCACACT -3'	213
GREM1	forward 5'- AAGGCACTTCCTGTTACTCTGC -3' reverse 5'- TACGACTGAGATGTCAGGGAGA -3'	256
GAPDH	forward 5'-GGGTGTGAACCACGAGAAATATGA-3' reverse 5'-AGCACCAGTGGATGCAGGGATGAT-3'	240

A computer program (Graph Pad Prism version 5.00 for Windows, Graph Pad Software, and San Diego California USA) was used for statistical analysis. The distribution of each quantitative parameter was estimated for normality by using the Shapiro-Wilk test. When a parameter was normally distributed, one-way ANOVA with Newman–Keuls post hoc test was used. The results were expressed as mean ± standard deviation (SD). The results of semi quantitative ICC score were compared using Kruskal–Wallis test and were expressed as the median (range). Where differences among the groups were detected, medians were compared using the Dunn's multiple-comparison test. P-values <0.05 were considered significant.

RESULTS AND DISCUSSION

In the present study, we used a mouse model of immune complex-mediated pathology induced by immunization with BSA. As we demonstrated earlier, [9] administration of BSA according to the described protocol resulted in immune cells activation, elevated level of ICs 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. Here we found that 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 (Figure 1a) that was accompanied by impairment of the meiotic maturation of oocytes (Figure 1b): the number of oocytes at metaphase I and metaphase II decreased significantly compared to that of control mice.

We also estimated in cumulus cells the expression levels of COX2, GREM1 and HAS2 genes which are related to mouse oocyte developmental competence. COX2 has been shown to facilitate cumulus expansion via prostaglandin E2 (PGE2) production. Mice lacking a functional COX2 gene have defects in ovulation, fertilization, decidualization and implantation. The relationship between oocyte quality and GREM1 expression is less clear. The regulation of BMP (bone morphogenic protein) signalling through GREM1 is thought to contribute to CCs expansion and therefore to the final maturation of oocytes. Another studied gene was HAS2. It encodes an enzyme responsible for the synthesis of hyaluronic acid and proteoglycan versican, which are important components of the cumulus matrix. The expression of investigated genes in the cumulus may give a direct assessment of the fertility potential of an individual oocyte. [11,12]

In control mice, the mRNAs encoding these genes were detected in all tested cumulus cell samples: PCR amplification of the reverse-transcribed mRNA yielded specific bands of 409, 213, and 256 bp, the expected sizes of HAS2, COX2 and GREM1 respectively. The

immunization with BSA resulted in significantly reduced mRNA expression levels for all the studied genes compared with control mice (Figure 1c), that indicate impaired cumulus expansion and therefore loss of oocyte quality. All together, our data indicate that systemic immune complex–mediated injury induced in mice by long term BSA immunization lead to disturbances in ovarian function.

PARP1 is generally believed to play a key pathological role in various immune-mediated diseases [1,2] but its involvement in immune complex induced tissue injury is poorly understood. Here we assessed PARP activity in cumulus cells by ICC detection of PAR - the product of this enzyme). ICC score for PAR level was significantly elevated in BSA-administered mice (Me, min-max: 0.31, 0.17-0.41) as compared with control (0.08, 0.04-0.19, $P<0.001$). Injections of 4-HQ to the immunized mice decreased ICC score to 0.12 (0.06-0.21), $p<0.05$ as compared with immunization. These results indicated that PARP is activated in mice with experimental immune complex induced tissue injury and that administration of 4-HQ according to the described treatment scheme was effective to substantially prevent PARP activation in this pathology.

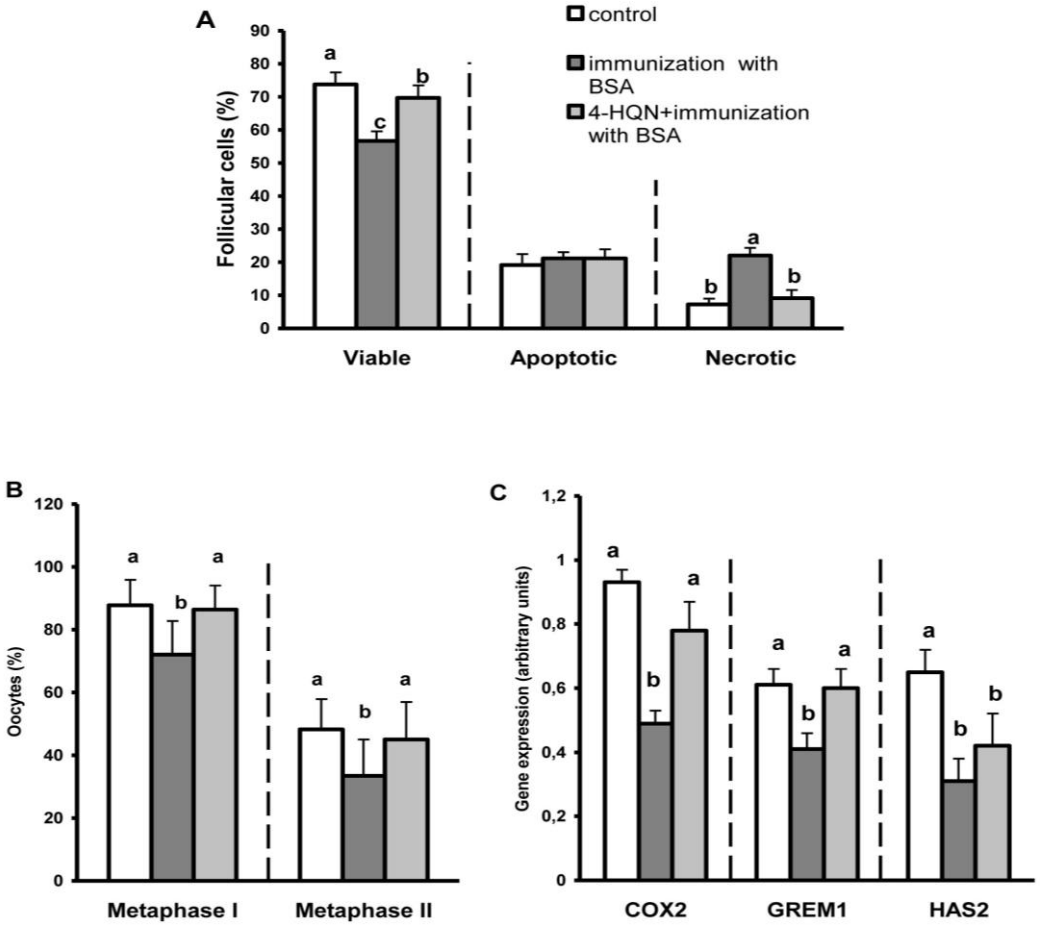

Figure 1: The effect of 4-HQN on the percentage of viable, apoptotic or necrotic follicular cells (A); on the percentage of oocytes with germinal vesicle breakdown - metaphase I and oocytes forming the first polar body - metaphase II (B) and on Cox2, Grem1 and Has2 genes expression in mice immunized with BSA. Control mice received saline. Data is present as mean±SD. Different superscripts indicate significant differences ($p<0.05$).

The treatment of the immunized mice with 4-HQ improved the meiotic maturation of oocytes at metaphase I and II in comparison to immunized mice (Figure 1b). Injections of 4-HQ to BSA treated mice significantly enhanced the expression of mRNA for COX2 and GREM1 compared with these levels in immunized group (Figure 1c). 4-HQ treatment also increased the HAS2 gene expression (by 34 %),

although this effect did not achieve statistical significance. Studied genes play an important role in follicular development and cumulus expansion and therefore in the development and maturation of oocytes. It was found that cumulus COX2, GREM1 and HAS2 expression correlates to oocyte quality, fertilization and embryo morphology. [11,12] Thus, PARP inhibition significantly improves ovarian function disturbances induced by immunization with BSA in mouse model of immune complex-mediated disease.

Today, it is established that over activation of PARP1 in response to extensive DNA damage causes depletion of NAD (+) and ATP which may lead to the energy failure, loss of cell membrane integrity and necrotic cell death. [2,3] This is one of the key mechanisms of PARP1 mediated exacerbation of immune inflammation since necrotic form of cell death is regarded as highly proinflammatory and immunogenic. The release of intracellular molecules (proteases, intracellular auto antigens and other inflammatory mediators as alarmines) through disrupted plasma membrane may further amplify the inflammatory and immune response, thus forming a pathogenic feedback loop. Therefore, the protective effect of PARP inhibition may be mediated, at least partially, through the attenuation of necrosis. Our data obtained in a mouse model of immune complex induced tissue injury confirmed this assumption. Here, we found that the treatment of immunized mice with 4-HQ improved granulosa cell viability compared with compared with BSA group (Figure 1a). Inhibition of PARP diminished necrosis, increased in response to BSA administration, while the percentage of apoptotic cells remained unchanged.

In addition to regulating necrosis, PARP1 can act as a co-activator of proinflammatory transcription factors such as NFκB and AP-1. Thus, inhibitors of PARP can prevent the expression of inflammatory mediators such as iNOS (inducible nitric-oxide synthase), cytokines, chemokines, and adhesion molecules. [1,3] But this mechanism of beneficial action of 4-HQ on immune complex-mediated ovarian pathology requires further clarification.

Since PARP1 over activation can induce a self-amplifying cycle of cell death, immune response and inflammation, inhibition of this enzyme might interrupt this vicious circle. Numerous studies demonstrated the protective action of PARP inhibition in various animal models of immune-mediated diseases. In this investigation, we also found for the first time that PARP inhibitor, 4-HQ, exerted a beneficial effect on experimental immune complex mediated-ovarian failure in mice, at least partially due to attenuation of necrotic cell death. All these findings make PARP1 an attractive target for the therapy of inflammatory disorders. Since PARP plays an important role in many physiological housekeeping processes (such as gene repair, transcription, cell cycling, mammalian oogenesis and folliculogenesis), caution should be used to avoid the possible side effects. [2,4,5] For that reason, PARP inhibitors may be particularly useful in the treatment of acute inflammatory disorders, but complete inhibition of PARP1 appears undesirable in chronic inflammatory diseases. [6] Mild inhibition would be the preferred method of action. [13]

Many endogenous and dietary compounds (such as purines, nicotinamide, some vitamins etc) have been shown to inhibit PARP1 in vitro and in vivo. Dietary flavonoids like quercetin, fisetin, and tricetin were identified as significant inhibitors of this enzyme. [13] So it has been suggested that naturally occurring modulators/inhibitors of PARP1 may be effective for prevention and therapy of chronic inflammatory diseases through dietary supplementation. [6] We suppose that inhibition of PARP1 by food-derived substances might attenuate immune-mediated disturbances of reproductive function in women that requires further

investigation.

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REFERENCES

1. Rosado MM, Bennici E, Novelli F, Pioli C. Beyond DNA repair, the immunological role of PARP-1 and its siblings. Immunology. 2013;139 (4): 428-37.
2. Bai P, Virág L. Role of poly (ADP-ribose) polymerases in the regulation of inflammatory processes. FEBS Lett. 2012; 586(21):3771-7.
3. Jog NR, Caricchio R. The Role of Necrotic cell death in the pathogenesis of immune mediated nephropathies. Clin Immunol. 2014; 153(2):243–53.
4. Qian H, Xu J, Lalioti MD, Gulle K, Sakkas D. Oocyte numbers in the mouse increase after treatment with 5-aminoisoquinolinone: a potent inhibitor of poly (ADP-ribosyl) ation. Biol Reprod. 2010; 82(5):1000-7.
5. Wei Q, Ding W, Shi F. Roles of poly (ADP-ribose) polymerase (PARP1) cleavage in the ovaries of fetal, neonatal, and adult pigs. Reproduction. 2013; 146(6):593-602.
6. Banasik M, Stedeford T, Strosznajder RP. Natural inhibitors of poly (ADP-ribose) polymerase-1. Mol Neurobiol. 2012; 46(1):55-63.
7. Makogon N, Voznesenskaya T, Bryzgina T, Sukhina V, Grushka N, Alexeyeva I. Poly (ADP-ribose) polymerase inhibitor, 3-aminobenzamide, protects against experimental immune ovarian failure in mice. Reprod Biol. 2010; 10(3):215-26.
8. Lytvynenko A, Pavlovych S, Makogon N, Yanchiy R. Effects of inhibitor of poly(adp-ribose)polymerase on the deposition of immune complexes and uterine contractility under systemic immune complex disorders in mice. Medical hydrology and rehabilitation. 2014; 12(1-4):15-21.
9. Pavlovich S, Litvinenko A, Makogon N, Martynova T, Bryzgina T, Yanchii R, et al. Characterization of mouse model of a systemic immune complexes mediated pathology. Reports of morphology. 2014; 20(2):496-500.
10. Tóth-Zsámboki E, Horváth E,Vargova K, Pankotai E, Murthy K, Zsengellér Z, et al. Activation of poly (ADP-ribose) polymerase by myocardial ischemia and coronary reperfusion in human circulating leukocytes. Mol Med. 2006; 12(9-10):221-8.
11. Uyar A, Torrealday S, Seli E. Cumulus and granulosa cell markers of oocyte and embryo quality. Fertil Steril. 2013; 99(4):979-97.
12. Fragouli E, Lalioti MD, Wells D. The transcriptome of follicular cells: biological insights and clinical implications for the treatment of infertility. Hum Reprod Update. 2014; 20(1):1-11.
13. Geraets L, Moonen HJ, Brauers K, Wouters EF, Bast A, Hageman GJ. Dietary flavones and flavonoles are inhi bitors of poly (ADP-ribose) polymerase -1 in pulmonary epithelial cells. J Nutr. 2007; 137(10):2190-5.

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