ACTION OF DANAZOL ON 5′-NUCLEOTIDASE ACTIVITY OF MOUSE PERITONEAL MACROPHAGES

V. V. Mit'kin, N. I. Volkov, T. Ya. Pshenichnikova, and G. T. Sukhikh

UDC 615.357.015.4.076.9

KEY WORDS: danazol; peritoneal macrophages; cyclic AMP.

Danazol, a derivative of 17-ethinyltestosterone, has been used in the treatment of endometriosis since 1967 [9]. The pharmacologic properties of danazol are well known: 1) suppression of secretion of gonadotrophin releasing hormone and/or of gonadotrophins; 2) direct interaction with endometrial androgen and progesterone receptors; 3) suppression of ovarian steroid production; 4) stimulation of estradiol and progesterone metabolism [4]. These properties determine the final effect of the drug, which is to inhibit growth of endometrial implants [13].

Recently new information has been obtained which confirms the role of immunologic mechanisms in the pathogenesis of endometriosis [1, 2]. It has been suggested that immunity factors may have a modulating action on growth of endometrial implants and on the development of the sterility which is associated with endometriosis [5]. In women with endometriosis a higher concentration of active peritoneal macrophages and lymphocytes is found than in normal women [11]. These cells can directly cause infertility, for it has recently been shown that secretions of active lymphocytes and macrophages (lymphokines and monokines) have a reversible effect on spermatozoal motility and development of the embryo [7].

The aim of this investigation is to assess the effect of danazol in vitro and in vivo on activity of mouse peritoneal macrophages. A characteristic sign of macrophage activation is a decrease in activity of 5′nucleotidase, a physiologically important regulator of cyclic AMP metabolism.

EXPERIMENTAL METHOD

C57BL/6 mice were used. Danazol in vivo was injected as a single dose of 0.5 ml subcutaneously. Control animals received 0.5 ml of a 150 mM solution of NaCl. The animals were killed 24 h later and peritoneal macrophages obtained by washing out the peritoneal cavity with 3-4 ml of medium 199 with heparin (5 U heparin to 1 ml medium). The cell suspension thus obtained was filtered through a Kapron filter and applied to plastic Petri dishes. The dishes were kept for 1.5 h in a CO_2 incubator (37°C). After incubation the dishes were washed five times with physiological saline to remove nonadherent cells. Adherent cells were removed and their concentration adjusted to 2 million cells/ml.

In vitro, a solution of danazol ("Stirling Winthrop Research"), made up in ethyl alcohol, was added to the monolayer of adherent cells in a final concentration of 10^{-4}-10^{-14} respectively. The alcohol concentration in the dish did not exceed 5%. After incubation for 5 h in the CO_2 incubator (37°C) the cells were washed with physiological saline and removed in order to determine their 5′-nucleotidase activity.

The principle of determination of 5′-nucleotidase [1, 3, 5] in peritoneal macrophages is based on the ability of this enzyme to hydrolyze 5′-nucleotides and, in particular, adenosine-5-monophosphate, with the formation of phosphoric acid, the level of which reflects enzyme activity. Considering that 5′-nucleotidase is a typical ectoenzyme and is located in the cytoplasmic membrane of the cell, activity of the enzyme was determined in a suspension of intact cells. Phosphorus was

All-Union Research Center for Health Care of Mother and Child, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 2, pp. pp. 177-178, February, 1992. Original article submitted January 15, 1991.

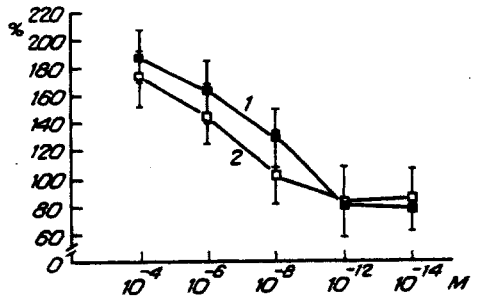

Fig. 1. 5′-Nucleotidase activity (in % of control) of peritoneal macrophages in response to danazol: ■) in vitro; □) in vivo.

determined by the addition of a mixture of molybdate reagent and ascorbic acid. The density of the color was measured on a "Labsystems" multiscanner at a wavelength of 620 nm (No. 7 filter).

Activity of the enzyme was expressed as a percentage of the control, taken as 100%.

EXPERIMENTAL RESULTS

The results showing the action of danazol in vivo are given in Fig. 1. They show that with an increase in concentration of the preparation activity of 5′-nucleotidase increased, to reach 174.6 ± 18.4% at a dose of 10^{-4} M. A concentration of danazol of 10^{-6} M, corresponding to a pharmacologic concentration of 600 mg/day when administered perorally to women, significantly stimulates peritoneal macrophage activity (by 148.3 ± 14.8%). Lower doses had no marked action on enzyme activity. An increase in activity of the enzyme is evidence of inhibition of macrophagal activity. Subcutaneous injection of danazol into mice thus leads after 24 h to a marked reduction of peritoneal macrophage activity.

The effect of danazol in vitro is also shown in Fig. 1. The character of its action was similar to that in vivo, i.e., an increase in activity of the enzyme took place with an increase in concentration. However, the degree of the response to danazol was more marked in this experiment. For example, in a dose of 10^{-6} M 5′-nucleotidase activity increased by 164.3 ± 21.7%, and in a dose of 10^{-4} M by 186.1 ± 24.3%. Thus a significant decrease in peritoneal macrophage activity took place.

Glucocorticoids and sex steroids have an effect both in vitro and in vivo on immunologic functions. Involvement of sex steroids in regulation of the immune system is demonstrated by the following data: 1) the degree of the immune response depends on sex; 2) gonadectomy and steroid therapy affect the immune response; 3) changes in the immune response during pregnancy; 4) cells of the immune system (lymphocytes) express receptors for steroid hormones [10].

The inhibitory effect of danazol on peritoneal macrophage 5′-nucleotidase activity thus revealed in vitro and in vivo may be of great importance in the treatment of endometriosis. It has been suggested that autoimmune disturbances play a significant role in the genesis of endometriosis [8]. The peritoneal fluid of patients with endometriosis contains more leukocytes (lymphocytes and macrophages) than that of women without endometriosis [11]. Endometrial cells, which are the possible antigenic source for activation of peritoneal lymphocytes and macrophages also are found in the peritoneal fluid. Activated macrophages, in turn, produce soluble factors (monokines), inducing differentiation of lymphocytes and production of the corresponding soluble factors (lymphokines). Lymphokines and monokines act reversibly on reproductive processes such as motility of spermatozoa and development of the embryo, and they may perhaps also play a key role in the mechanism of sterility of unknown genesis [6, 12].

The pharmacologic hormonal effects of danazol are well known. Danazol is also used in the treatment of autoimmune diseases such as systemic lupus erythematosus, thrombocytopenic purpura, and angioneurotic edema [3, 14]. However, the immunologic aspects of the action of danazol have not been finally elucidated.

Our findings confirm the possibility of new mechanisms of action of danazol in the treatment of endometriosis. First, the immunosuppressive effect (S) of danazol may influence autoimmune mechanisms which play an important role in the etiology and clinical picture of endometriosis. Second, danazol, by depressing or blocking activation of cells in the peritoneal fluid by endometrial antigens, inhibits secretion of leukocytic monokines and lymphokines. It is therefore

possible that danazol may increase fertility of patients with endometriosis through its direct suppressive action on peritoneal macrophages.

Our observations also indicate that the method of determination of 5'-nucleotidase activity in peritoneal macrophages, together with clinical evaluation of the therapeutic action of danazol, can be used to identify the optimal daily dose of this preparation when used in the treatment of endometriosis in women with sterility.

LITERATURE CITED

1. V. V. Mit'kin, V. I. Kulakov, and G. T. Sukikh, Akush. Gin.
2. G. T. Sukikh, N. I. Volkov, and I. D. Ionov, Diagnosis and Treatment of Childless Marriage [in Russian], Moscow (1988), pp. 122-127.
3. Y. Ahn, W. Harrington, S. Simon, et al., New Engl. J. Med., **308**, 1396 (1983).
4. R. Barbieri and K. Ryan, Am. J. Obstet. Gynecol., **141**, 453 (1981).
5. D. Bartosik, Sem. Reprod. Endocrinol., **3**, 329 (1985).
6. A. El-Roeiy, W. Dmowsky, N. Gleicher, et al., Fertil. Steril., **50**, 864 (1988).
7. H. Fakih, B. Baggett, G. Holtz, et al., Fertil. Steril., **47**, 213 (1987).
8. N. Gleicher, A. El-Roeiy, E. Confino, et al., Obstet. Gynecol., **70**, 115 (1987).
9. R. Greenblatt, W. Dmowski, V. Mahesh, et al., Fertil. Steril., **22**, 102 (1971).
10. C. Grossman, Science, **227**, 257 (1985).
11. A. Haney, J. Muscato, and J. Weinberg, Fertil. Steril., **35**, 696 (1981).
12. J. Hill, F. Haimovici, J. Politch, et al., Fertil. Steril., **47**, 460 (1987).
13. J. Hill, R. Barbieri, and D. Anderson, Fertil. Steril., **48**, 414 (1987).
14. R. Mylvaganam, Y. Ahn, W. Harrington, et al., Clin. Immunol. Immunopathol., **42**, 281 (1987).

EFFECT OF EXPERIMENTAL ULTRAVIOLET IRRADIATION OF BLOOD ON IMMUNITY

V. V. Safonov and D. A. Voevodin

UDC 615.38.015.2:615.831.4].015.4:612.017.1].076.9

KEY WORDS: blood; ultraviolet; irradiation.

Data on the effect of ultraviolet irradiation of blood (UVIB) on the state of immunity are contradictory, and range from immunostimulation [5] to immunosuppression [1]. The view also is held that UVIB has an immunocorrective action [4], and this is evidently dependent on the initial state of the immune system. There is no single method of carrying out UVIB. Although generally similar, they can differ greatly both in the quantity of blood irradiated and in the frequency and duration of the irradiation sessions, and this again is evidently connected with differences in the design of the apparatus used for UVIB [3]. Although they share the same working principle, they differ in the configuration of the cuvettes and the distance from the source of light to the blood. Since UV radiation penetrates into blood for a distance of only 50 μ, the quantity of blood actually undergoing photomodification depends on the area of the irradiated surface. The results of the use of UVIB also depend largely on the distance between the blood and the source of radiation, for the spread of UV radiation falls away sharply during passage through air [2].

Clinic for Pediatric Surgery, Anesthesiology, and Resuscitation, Second Moscow Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 2, pp. 178-179, February, 1992. Original article submitted July 20, 1991.