Effects of Afala and Antiestrogen ICI 182,780 in the Model of Hormone-Dependent Prostate Inflammation

J. Bernoulli*, Y. Konkol*, H. Vuorikoski*,, and E. Yatkin*****

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In the hormone-dependent prostate inflammation model induced by implantation of slow-releasing pellets (50 mg testosterone and 5 mg estradiol) to Noble male rats, intragastric administration of Afala at a dose of 7.5 ml/kg for 18 weeks reduced the number of inflamed prostatic acini. The effect of afala was comparable with that of antiestrogen ICI 182,780 (3 mg/kg subcutaneously twice a week for 18 weeks). Prolonged treatment with hormones in high doses induced severe inflammation of the prostate tissue, which was not terminated by the test preparations. As differentiated from the antiestrogen ICI 182,780, afala did not induce body weight gain and decrease in pituitary weight in experimental animals in comparison with the control group.

Key Words: *afala; hormone-dependent prostate inflammation; abacterial prostatitis; ICI 182,780*

Chronic abacterial prostatitis/chronic pelvic pain syndrome (CAP/CPPS) is a common disease of men of all age groups. It is manifested by pain in the groin and pubic area, perineum, and genital organs. This disease is characterized by the presence of lymphocytes and macrophages in the prostate stroma and intraepithelial space, as well as of neutrophils in prostate acini [10]. Some authors reported that prostate inflammation can be related to other diseases, including benign hyperplasia [9,13].

The etiology and pathogenesis of CAP/CPPS are poorly understood. Much attention was paid to studying the pathogenetic factors of prostatitis [11]. Sex hormones play a particular role in the development of this disease [12]. Previous studies have demonstrated that combined administration of testosterone and estradiol to intact male rats for 6 weeks was followed by the development of prostate inflammation [6]. The severity of this disorder increased with increasing in the duration of hormone administration to 13 weeks. The symptoms of prostate inflammation in rats resembled symptoms of exacerbation of chronic prostatitis in men [6,15].

The general health-improving medicines, immunomodulatory agents, and nutraceuticals are used in the therapy of CAP/CPPS [7,8]. Low clinical efficacy of existing therapeutic agents necessitates the development of new approaches to pharmacotherapy of this pathology [5]. Afala contains affinity-purified antibodies to the prostate-specific antigen (PSA) in a release-active form. Good effectiveness of afala was demonstrated in preclinical studies on the model of acute and chronic prostatitis in rats, as well as in clinical studies in patients with chronic bacterial prostatitis [1-3]. Release-active antibodies can produce a direct modifying effect on spatial configuration of specific antigen, which contributes to variations in its physicochemical and biological properties [4].

Here we studied the effect of afala on the development of inflammation in the prostate induced by testosterone and estradiol.

*Pharmatest Services Ltd; **Montisera Ltd, Turku; ***University of Turku, Finland. ***Address for correspondence:*** nauka@materia-medica.ru; jenny.bernoulli@pharmatest.com. J. Bernoulli

MATERIALS AND METHODS

Experiments were performed on adult male Noble rats weighing 320.8±28.0 g. The animals were divided into 4 groups (10 per a group). The pellets providing a slow release of testosterone (T; 50 mg) and estradiol (E_2; 5 mg) and placebo pellets (Innovative Research of America, Inc.) were implanted to 13-14-week-old rats under isoflurane anesthesia. The pellets were inserted in subcutaneous pockets formed over the scapular area through a 1-2-cm cuts. The pellets were replaced twice by identical ones over 18 weeks. Group 1 animals received a placebo implant and distilled water (7.5 ml/kg) through a probe for 18 weeks. Group 2 (control) rats received the implant for a slow release of T and E_2 and distilled water (7.5 ml/kg) through a probe. Group 3 rats received a T+E_2 implant and were treated with afala (7.5 ml/kg) through a probe. Group 4 animals received T+E_2 implant and were subcutaneously injected with the antiestrogen ICI 182,780 (3 mg/kg) twice a week.

The body weight gain was recorded during the period from the first implantation to CO_2 euthanasia.

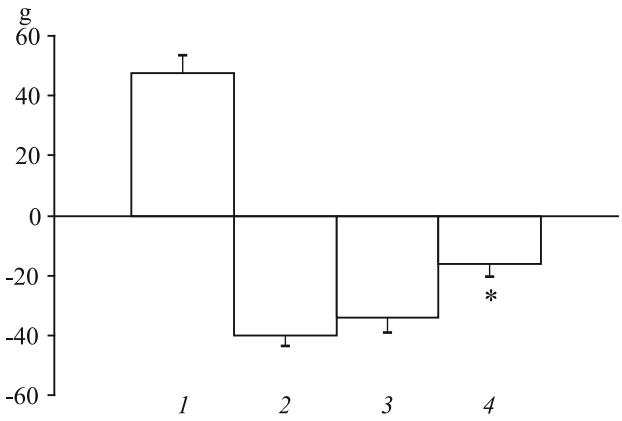

Fig. 1. Body weight changes in experimental animals in the period between the first implantation and euthanasia. Here and in Fig. 3: placebo group (*1*), control group (*2*), afala group (*3*), and ICI 182,780 group (*4*).

The seminal vesicles, urethra, prostate, and pituitary gland were isolated and weighted during autopsy. The samples were prepared for a histological study. Quantitative analysis of prostate inflammation was based

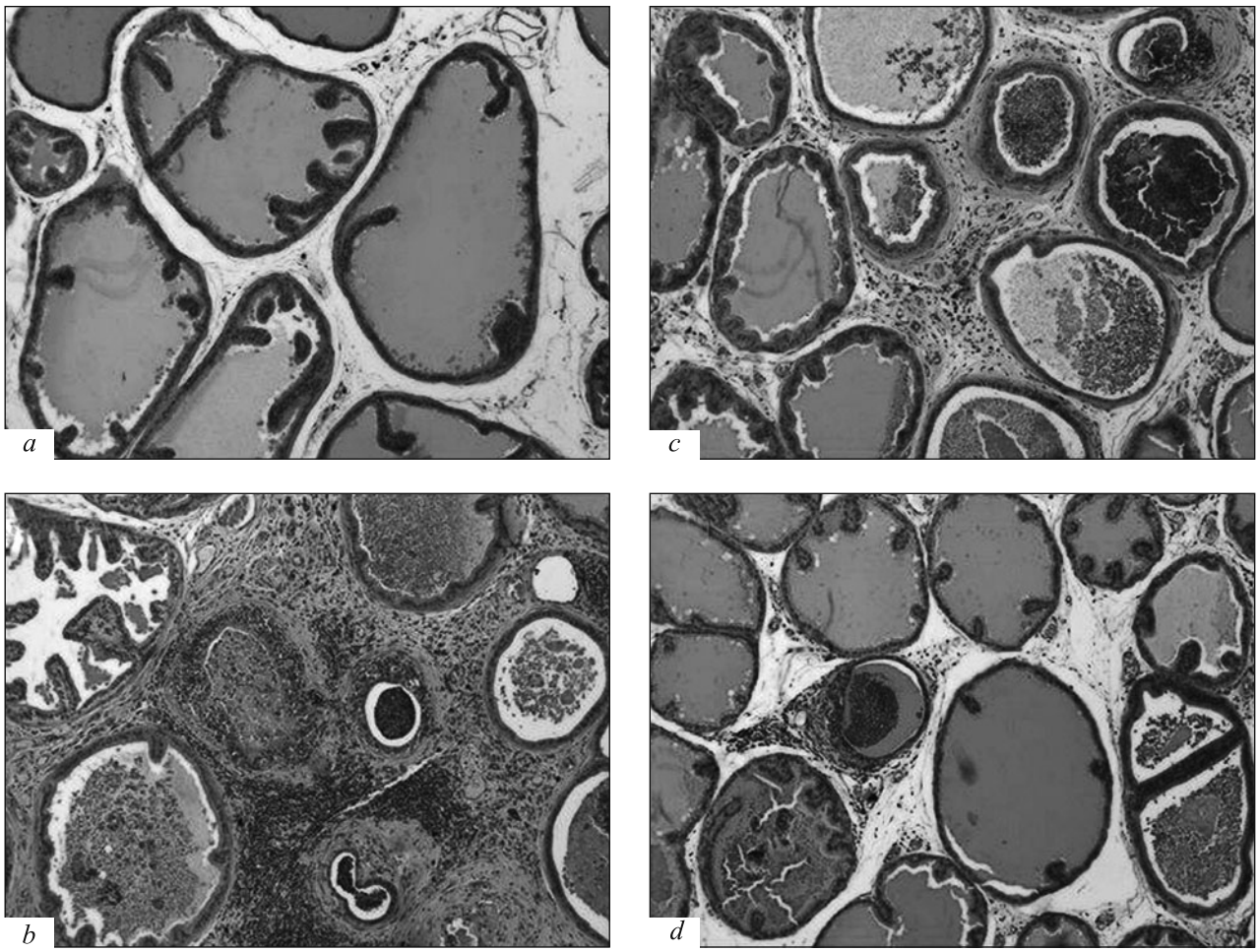

Fig. 2. Prostate sections from experimental rats. Staining with hematoxylin and eosin (×40).

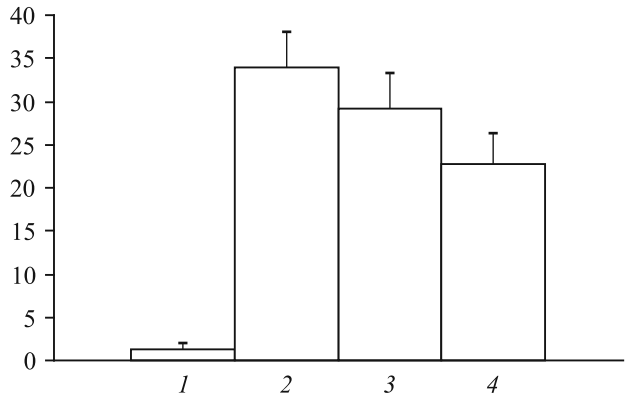

Fig. 3. Effect of study drugs on the number of inflamed acini.

on visual evaluation of its severity (4-point scale) and calculation of the mean number of inflamed acini [15]. Three to the four sections that contained dorsolateral prostatic lobes were selected for hematoxylin and eosin staining.

The normality of data distribution for body weight gain and increase in the weight of organs was verified by Kolmogorov–Smirnov test. One-way ANOVA and Student–Newman–Keuls post-hoc test were used in case of normal distribution. If the distribution differed from normal, Kruskal–Wallis one-way analysis of variance and Dunnett's post-hoc test were used. Statistical processing of the data was performed with SigmaStat 3.5 software (Systat Software Inc., Richmond, California). The plots were constructed with GraphPad Prism 4.00 software (GraphPad Software).

RESULTS

Significant delay in body weight gain (by 22.8%) was observed in group 2 (control) rats in comparison with the placebo group ($p<0.05$). Afala had no effect on the body weight of animals, *i.e.* no significant differences were revealed between controls and afala-treated animals (group 3). Administration of ICI 182,780 was followed by a significant increase in the body weight (by 5%, $p<0.05$ compared to the control; Fig. 1).

The relative weights of the pituitary gland, seminal vesicles, and prostate in control rats were higher than in animals of the placebo group (by 6.3, 3.1, and 2.4 times, respectively; $p<0.05$). These differences were probably related to the effect of E_2 and T [15]. The weight of these organs in afala-receiving rats practically did not differ from that in control specimens. ICI 182,780 had no effect on the weight of the seminal vesicles and prostate, but significantly decreased the relative weight of the pituitary gland (by 1.9 times; $p<0.05$).

Histological study revealed no signs of inflammation in animals of the placebo group (Fig. 2, *a*). In control rats, the inflammatory reaction was manifested in stromal infiltration with lymphocytes and mast cells. Neutrophils were found inside the acini. The intraepithelial space was shown to contain macrophages (Fig. 2, *b*). The severity of inflammation was lower in animals receiving ICI 182,780 and afala (Fig. 2, *c*, *d*). Quantitative analysis showed that afala and ICI 182,780 had little effect on the degree of inflammation, but a tendency to a decrease the number of inflamed acini was observed (compared to the control; Fig. 3).

Our results indicate that administration of ICI 182,780 is followed by an increase in the body weight and decrease in the weight of the pituitary gland in animals (as compared to control specimens). Therefore, this agent possesses antiestrogen properties. Afala had no effect on the body weight and weight of examined organs, which is consistent with the results of previous studies [3]. Afala and ICI 182,780 tended to decrease the number of inflamed acini (as compared to that in control specimens), which agrees with published data on anti-inflammatory activity of these agents [3,14]. It should be noted that in our study high doses of hormones were administered for a long period (18 weeks), which resulted in the development of severe inflammation. Correction of this state will evidently require the use of the study drugs in high doses.

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