Modification of Sulpiride Model of Benign Prostatic Hyperplasia for Evaluation of the Effectiveness of Drug Therapy

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Specific effects of ultra-low doses of antibodies to prostate-specific antigen obtained from laboratory rats of late reproductive age with sulpiride model of benign prostatic hyperplasia were compared with the results of clinical application of the preparation. Clinical reproducibility of experimental data was proven, which suggests that the developed model is adequate for pilot testing of the effects of pharmacotherapy of this disease.

Key Words: *modification of the model for benign prostatic hyperplasia; pharmacological effects*

Benign prostatic hyperplasia (BPH) is one of the most common urological diseases of elderly men, although it may also occur between the ages of 40-50 years [4]. Among the methods of treatment for this disease, α1-adrenergic receptor blockers, inhibitors of 5α-reductase, are widely used [6]. These drugs are characterized by high clinical efficacy, but at the same time they produce various side effects, including hypotension, dizziness, erectile dysfunction, *etc*., which limits their use and necessitates the search for new drugs for the treatment of this pathology [3]. The latter requires the use of experimental models. Morphological signs of BPH in rats can be caused by injections of estradiol-17 and testosterone within 1-3 weeks after castration [10], and by prolonged administration of sulpiride causing hyperprolactinemia [13]. The latter model is most often used. Prolactin mediates the proinflammatory effect of estradiol on the prostate, so the model of BPH caused by hyperprolactinemia adequately reflects hormone-dependent inflammation of the prostate [12]. Long-term administration of sulpiride causes BPH in the lateral lobe of rat prostate, which is primarily affected by this pathology. There are different modifications for this model. Thus, BPH may be reproduced in young rats of reproductive age via administration of sulpiride within one month [2]. Introduction of sulpiride to castrated animals (against the background of substitutive administration of androgens) also causes hyperplasia of the lateral prostate lobe in rats [13]. The peculiarity of the sulpiride BPH model used in this pilot study lies in the fact that animals were injected with sulpiride at the late reproductive age. The animals of this age group were chosen because the incidence of BPH increases with age. Therefore this modification is most appropriate for its possible clinical application.

We studied the effects of ultra-low dose antibodies to prostate-specific antigen (ULD of antibodies to PSA) with BPH on this modified model compared to that of patients with symptomatic BPH in stages I and II. The choice of the drug for the study stems from the

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fact that ULD of antibodies to PSA are capable to adjust the balance of growth factors in prostate tissue [7].

The aim of the work was to study the prognostic significance of the sulpiride BPH model in rats of late reproductive age for assessment of clinical effectiveness of ULD of antibodies to PSA.

MATERIALS AND METHODS

Experiments were carried out on 10-month-old male Wistar rats (n=30). The animals were obtained from the nursery of the Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences (certificate available). The animals were kept according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). For obtaining morphological characteristics of BPH, male rats were injected with sulpiride (eglonil; Synthelabo Group) in a dose of 40 mg/kg daily for 2 months (subcutaneously) [13]. Preliminary experiments have shown that sulpiride administered to rats of the late reproductive age for a shorter period caused the appearance of morphological signs of BPH, but did not significantly increased the weight of the lateral prostate lobe and its mass index. We thought it important to obtain reliable increase in this index, because the existence of a correlation between the size of the prostate and the severity of lower urinary tract symptoms (LUTS), a clinical manifestation of BPH in humans, was proven [8].

The animals were divided into 3 groups: group 1, background (intact animals); group 2, control (sulpiride daily intraperitoneally in a dose of 40 mg/kg and the solvent daily intragastrically in a dose of 0.5 ml/100 g body weight for 60 days); group 3, sulpiride+ULD of antibodies to PSA (daily intragastrically in a dose of 0.5 ml/100 g body weight for 60 days). Two months after the experiment, the animals were weighed to determine the mass indexes of the prostate and then decapitated after ether anesthesia. For morphological analysis, the lateral lobe of the prostate was chosen, because according to previous findings [13] sulpiride even in long-term application does not affect the morphology of the anterior and posterior lobes. The lateral lobe of the prostate was prepared, weighed, and its mass index was determined.

For histological study, the lateral lobe of the gland was fixed in 10% formalin and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin. The number of points corresponding to the main structural components of the prostate (epithelium of the terminal parts of the ducts, lumen, and interstitium) per standard area was counted on stained slides using computer-aided graphic analysis. Then the percentage of given structural components was calculated [1].

In clinical trial of preparation effectiveness, 132 male patients (63.9±0.6 years) with stages I and II symptomatic BPH and disease history of 5.3±0.3 years were examined. The diagnosis of BPH was confirmed by transrectal ultrasonography. The patients were selected for the study on the basis of the following criteria: IPSS (International Prostate Symptom Score) 8-20 points, maximum urinary flow rate 5-15 ml/sec, prostate volume more than 25 cm^3, residual urine volume 150 ml, serum level of total prostate antigen <4 ng/ml. The study did not include patients who underwent surgery for prostate or bladder, as well as patients with severe concomitant diseases.

All patients received ULD of antibodies to PSA (2 tablets 4 times a day *per os* as monotherapy for 16 weeks). The effect of treatment was assessed after 16 weeks of therapy by IPSS, uroflowmetry, and transrectal ultrasound examination [8].

Statistical processing of obtained experimental and clinical data was performed using parametric Student's t test and nonparametric Mann–Whitney test.

RESULTS

Two months after the start of sulpiride administration to rats of the late reproductive age, morphological features of adenomatous hyperplasia in the lateral lobe of the prostate were reproduced in all animals. The diagnosis of BPH was based on revealed proliferation of epithelial cells in the acini with the formation of papillary structures (Fig. 1, *a*, *b*). Sometimes, epithelial papillae filled the entire lumen of the acinus, and the epithelium became cuboid. The layers of connective tissue between the terminal parts were widened. In addition, focal cellular infiltration of the stroma and the appearance of terminal segments with enlarged lumen filled with neutrophils were observed. The area of epithelium in terminal parts increased by 40%; the lumen of acini decreased by 30%, and the area of interstitial tissue increased by 20% compared to the background values (Fig. 2). The weight of lateral lobe of the prostate and its mass index increased by 1.9-2.0 times, respectively.

Administration of ULD of antibodies to PSA against the background of sulpiride treatment prevented the development of BPH symptoms (Fig. 1, *d*). Histological study revealed atrophy of the prostate epithelium. Terminal units containing white blood cells were observed in few cases. The area of epithelial structures significantly decreased by 28% and the area of the lumen in terminal units increased by 30% compared to the control (sulpiride, Fig. 2). The area of interstitial tissue tended to increase, but the differences were insignificant. The weight and mass

index of the prostate decreased by 42% and 38%, respectively. These parameters did not significantly differ from those in intact animals. This allows us to conclude that ULD of antibodies to PSA prevent the development of BPH caused by sulpiride in animals of the late reproductive age. The data obtained indicate high efficiency of the drug on this model.

Clinical manifestations of BPH in humans are LUTS considerably impairing the quality of life in male patients [8,9]. In ~50% patients with BPH treated with ULD of antibodies to PSA the severity of LUTS decreased by the 16th week of treatment. IPSS decreased by 58%, QoL index decreased by 46%, the maximum volume rate and mean urinary flow rate increased by 45% and 38%, respectively, residual urine volume decreased by 42% (Fig. 3). These findings attest to high efficiency of ULD of antibodies to PSA in patients with stage I and II symptomatic BPH. It should be noted that according to international recommendations [11], not medical treatment of BPH, but dynamic monitoring may be prescribed for patients with laboratory parameters corresponding to those after treatment with ULD of antibodies to PSA (Fig. 3).

It is known that prostate enlargement is a clinical manifestation of BPH [5]. In patients with BPH treated with ULD of antibodies to PSA, a statistically significant reduction in prostate volume was detected (Fig. 3). This obviously reduced the severity of LUTS. Based on experimental data, we can conclude that reduction of the prostate volume in patients with BPH is apparently a result of reducing the area of epithelial structure hyperplasia caused by administration of ULD of antibodies to PSA.

Comparison of the results of evaluation of drug efficacy on sulpiride BPH model in animals of the late reproductive age with those in patients with this pathology drove us to a conclusion that administration of the drug to animals prevented the development of

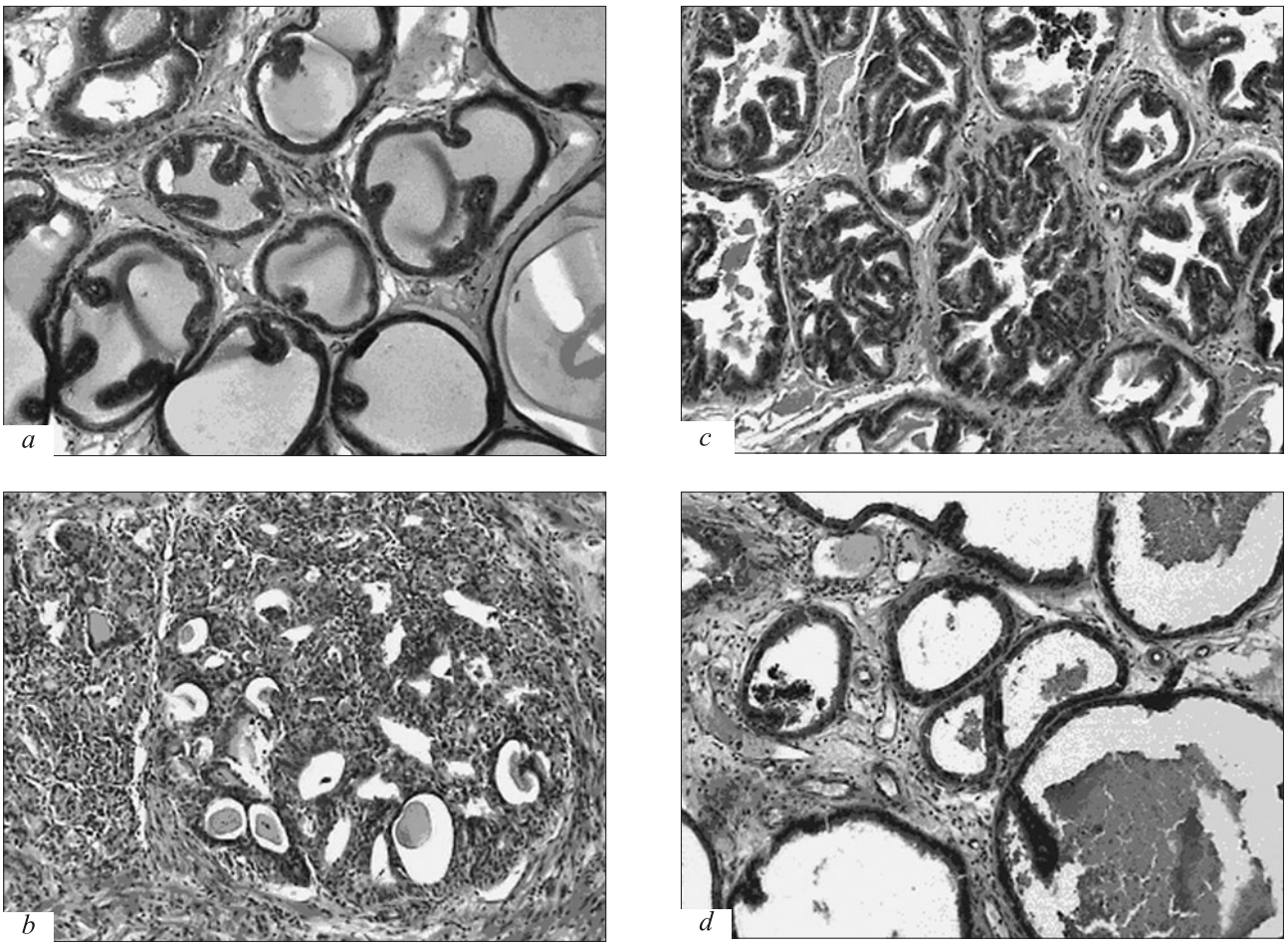

Fig. 1. Morphology of the prostate in rats of late reproductive age during administration of sulpiride and ULD of antibodies to PSA. Hematoxylin and eosin staining, ×200. *a*) lateral lobe of the normal prostate gland (intact animal); *b*) lateral lobe of prostate gland 2 months after administration of sulpiride, nodular hyperplasia of the prostate epithelium; *c*) lateral lobe of rat prostate gland 2 months after administration of sulpiride, papillary proliferation of prostate acini; *d)* lateral lobe of rat prostate gland 2 months after administration of sulpiride and ULD of antibodies to PSA, atrophy of the glandular epithelium.

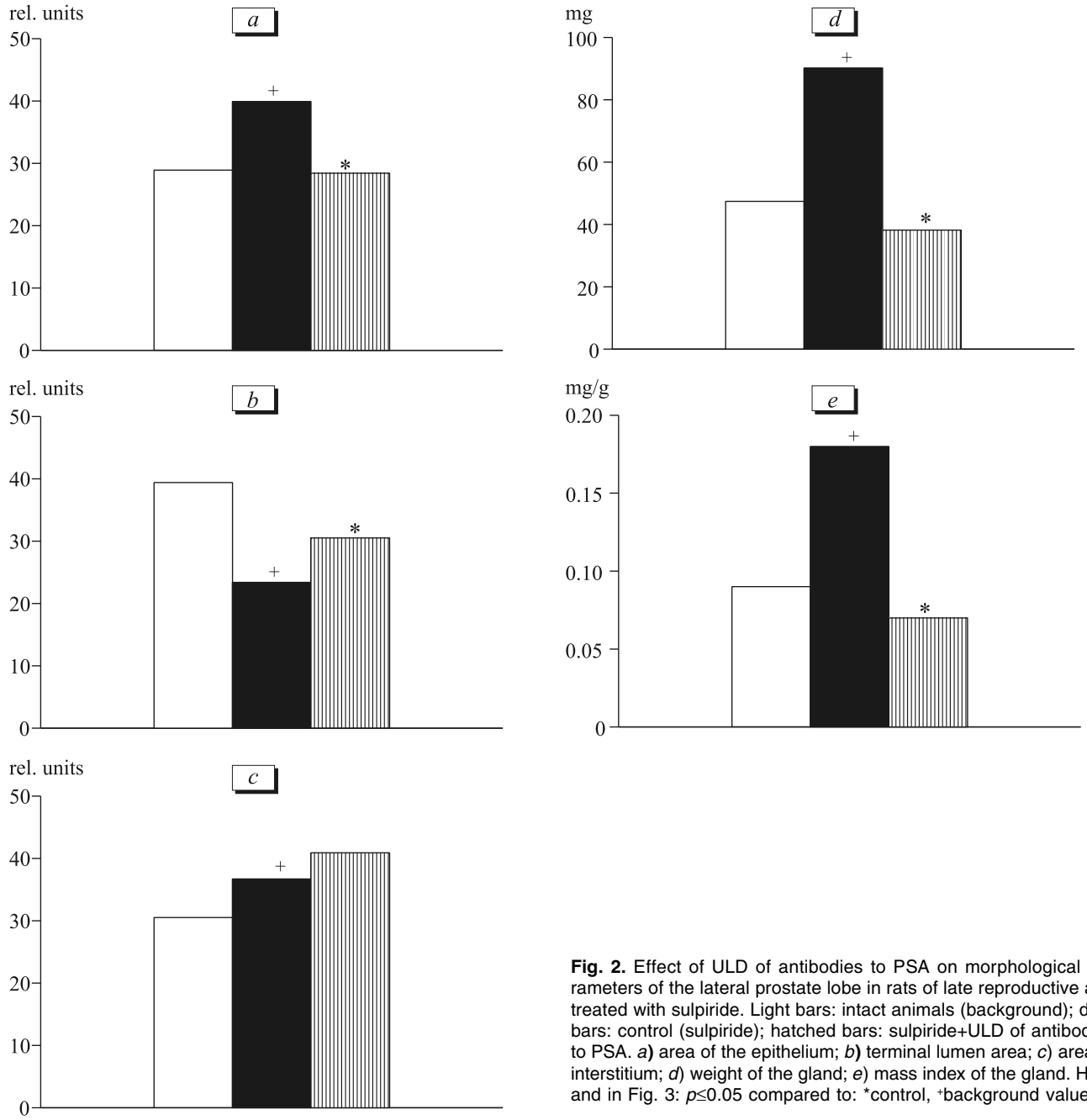

Fig. 2. Effect of ULD of antibodies to PSA on morphological parameters of the lateral prostate lobe in rats of late reproductive age treated with sulpiride. Light bars: intact animals (background); dark bars: control (sulpiride); hatched bars: sulpiride+ULD of antibodies to PSA. *a***)** area of the epithelium; *b***)** terminal lumen area; *c*) area of interstitium; *d*) weight of the gland; *e*) mass index of the gland. Here and in Fig. 3: $p \leq 0.05$ compared to: *control, +background values.

BPH. Laboratory parameters in patients treated with ULD of antibodies to PSA suggest that no drug therapy is required in these cases. The latter fact can be interpreted as arrest of the pathological process. Thus, the sulpiride BPH model in animals of the late reproductive age has a high predictive value in assessing the effectiveness of ULD of antibodies to PSA for patients with this pathology.

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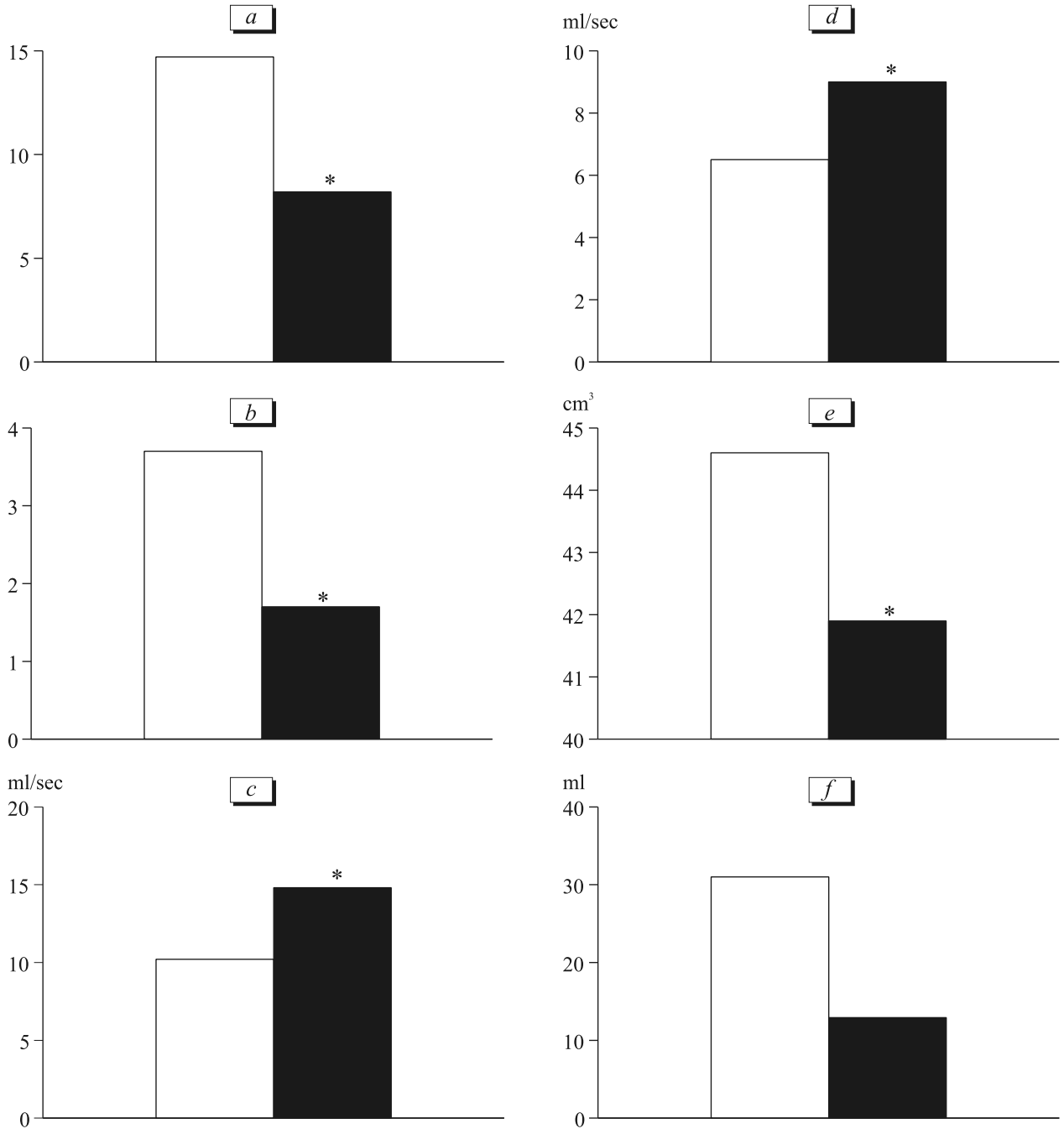

Fig. 3. Dynamics of changes in the studied parameters in patients with BPH receiving ULD of antibodies to PSA and treatment efficacy. Light bars: initial parameters; dark bars: parameters after 16-week treatment. *a*) total score IPSS; *b*) Quality of Life Index (QoL); *c*) maximum urinary flow rate; *d*) mean urinary flow rate; *e*) prostate volume; *f*) residual urine volume.

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