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Morphological and Biochemical Characteristics of Prostate Hyperplasia during Sulpiride Treatment

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We studied morphological changes in the prostate ventral lobe, proliferative activity of the epithelium in prostate acini, and the levels of prolactin and prostate-specific antigen in the blood serum of Sprague-Dawley rats after repeated injections of sulpiride in a dose of 40 mg/kg over 30 and 60 days and in 10 and 30 days after withdrawal. Morphological and morphometrical analysis of hyperplastic changes in the prostate ventral lobe was performed. Ki-67$^+$ proliferating epithelial cells in the acini were counted. The dynamics of serum concentrations of prolactin and prostate-specific antigen was evaluated by ELISA. Morphological and morphometrical analysis and evaluation of the content of Ki-67$^+$ cells demonstrated epithelium hyperplasia in the prostate ventral lobe after sulpiride treatment for 30 or 60 days and in 10 days after withdrawal, but serum level of prostate-specific antigen did not differ from the control. After 60-day sulpiride treatment and in 30 days after withdrawal, pronounced hyperplastic changes of prostate and elevated concentrations of prostate-specific antigen (but not prolactin) were observed. Thus, administration of sulpiride (40 mg/kg) to Sprague-Dawley rats for 60 days allows, by morphological criteria and serum level of prostate-specific antigen, to model stable hyperplastic changes in the prostate corresponding to benign prostatic hyperplasia in humans.

Key Words: *prostate; hyperplasia; rat; prolactin*

Benign prostatic hyperplasia (BPH) is a progressive neoplastic disease; its prevalence correlates with age [4]. According to epidemiological data; the incidence of BPH increases with age by 10% every year and affects more than 80% subjects aging above 80 [12]. The development of new therapeutic approaches requires deep understanding of the BHP pathogenesis; this, in turn, requires detailed characteristic of experimental models used in preclinical studies. BHP modeling is based on reproduction of hormone imbalance, in particular hyperprolactinemia and hyperandrogenemia. It was shown on hyperandrogenemia model that course administration of testosterone in high doses to rats led to epithelium hyperplasia in the ventral lobes of prostate gland (PG) similar to morphological changes in BPH in humans [2,3,11]. BHP modeling by treatment with sulpiride (blocker of D2 dopamine receptors) is based on stimulation of prolactin secretion in the pituitary, which in turn leads to hyperplasia of acinar epithelium in PG. Dopamine is the main regulator of prolactin synthesis in the pituitary in humans. Activation of D2 receptors on mammotropic cells of hypophysis by dopamine leads to inhibition of adenylate cyclase and cAMP-dependent protein kinase A, and finally to a decrease of prolactin synthesis [13]. Hyperprolactinemia induced by blockers of dopamine receptors suppresses secretion of gonadotropins, *e.g.* luteinizing hormone and sex steroids [15]. Prolactin regulates zinc uptake, citrate synthesis, and expression of androgen receptors and cathepsin D [10]. The interaction of prolactin with its receptors in PG acinar epithelium activates numerous signal pathways and molecules, including transcription activators STAT3

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and STAT5 stimulating production of cyclin D1 regulating cell transition from G1 to S phase of the mitotic cycle. Prolactin stimulates mitogen-activating protein kinase (MAPK), which also stimulates epithelium proliferation in PG. Hyperprolactinemia increases the expression of prolactin receptors and, as a consequence, promotes STAT3- and MAPK-dependent hyperplasia of PG epithelium [9,14,15].

It is known that the level of prolactin in the blood serum in men and laboratory male animals increases with age. Hence, the model of sulpiride-induced BPH based on induction of hyperprolactinemia, is the most adequate to BPH in humans [1]. Different administration schemes and doses of sulpiride are used for BPH modeling [1,6]. In most studies, sulpiride is administered in a dose of 30 or 40 mg/kg over 30 days. At the same time, there are no published data on reversibility of hyperplastic changes in PG, which is very important for preclinical evaluation of new drugs.

The aim of our study was to compare hyperplastic changes in PG and serum levels of prolactin and prostate-specific antigen (PSA) during course administration of sulpiride for 30 and 60 days and at different terms after withdrawal.

MATERIALS AND METHODS

The experiment was conducted on adult male Sprague-Dawley rats (n=32; initial body weight 180-210 g; age 50 days). All experimental manipulations were conducted according to directive 2010/63/EU (article 6) of European Parliament and the Council of the European Union on the Protection of Animals used for Scientific Purposes. The animals were housed in a vivarium at 23±3°C, humidity 35-65% and had unrestricted access to drinking water and food.

For BPH modeling, Eglonyl (Sulpiride) was intramuscularly injected to rats of the experimental group (4 groups, 4 rats per group) in a dose of 40 mg/kg for 30 and 60 days. The animals were sacrificed in 10 and 30 days after the end of drug administration by Zoletil overdose (Virbac Sante Animale). The animals of the control groups (4 groups, 4 rats per group) were sacrificed at the same terms as experimental rats.

The blood was taken from cervical veins, the serum was prepared and stored at -70°C for one month. Prolactin and PSA concentrations in the serum were measured by ELISA using commercial kits (Cloud-Clone Corp). Optical density was measured on an Anthos-2010 photometer with filters (400-750 nm) and ADAP+ software.

PG was isolated and placed in Bouin fixative for 24 h, then dehydrated in increasing concentrations of ethanol and xylene, embedded in paraffin, sliced on a microtome, and stained with hematoxylin and eosin. On histological sections, morphological changes in the ventral PG lobe were qualitatively evaluated. The relative volume of acini and stroma, acinar epithelium, and lumens were evaluated morphometrically (by point counting).

Proliferating cells in the epithelium of acini were visualized by immunofluorescence using primary anti-KI-67 antibodies (Thermo Scientific) and secondary antibodies conjugated with immunofluorescence marker Alexa Fluor 488 (Life technologies); the nuclei were poststained with DAPI. The number of Ki-67$^+$ cells (per 1000 μ^2) in the epithelium of acini were counted using ImageJ 1.50e software.

Statistical analysis was performed using Statistica 10.0 software (StatSoft, Inc.). After testing normality of data distribution using the Kolmogorov—Smirnov test, nonparametric statistics was applied. The data were presented as median (Me) and interquartile range (LQ; UQ). Significance of differences between the groups was evaluated by Kruskal—Wallis and Connover test. The differences were significant at p<0.05.

RESULTS

Histological analysis of the ventral PG lobe from rats treated with sulpiride for 30 and 60 days and sacrificed on days 10 and 30 after sulpiride withdrawal, numerous epithelial folds and acini lined with high cylindrical epithelium revealed. After 60 days of sulpiride administration and in 10 and 30 days after withdrawal, epithelial folds in acini markedly reduced their lumens.

After administration of sulpiride for 30 days and in 10 and 30 days after withdrawal, the volume fraction of acini, stroma, epithelium, and acinar lumens, as well as serum levels of prolactin and PSA did not differ from the control group (Tables 1, 2, Fig. 1). However, the number of Ki-67$^+$ cells in the acinar epithelium increased in comparison with the control (Table 1).

Morphometrical analysis revealed hyperplastic changes in PG after administration of sulpiride for 60 days and in 10 and 30 days after withdrawal. The volume fractions of acini and their epithelium significantly increased and volume fraction of the stroma and acinar lumens decreased (Table 1). The number of Ki-67$^+$ cells in the acinar epithelium was considerably higher than in the control group (Table 1, Fig. 2). In 10 and 30 days after sulpiride withdrawal, serum level of prolactin was unchanged (Table 2), while PSA level increased in comparison with the control group (Fig. 1).

Thus, administration of sulpiride in a dose of 40 mg/kg for 60 days to Sprague-Dawley rats led to stable hyperplastic changes in PG characterized by in-

TABLE 1. Morphometric Parameters of Structures in the Ventral Lobe of PG and the Number of Ki-67$^+$ Cells in the Acinar Epithelium after Administration of Sulpiride (40 mg/kg) for 30 and 60 days and in 10 and 30 Days after Withdrawal (Me (LQ-UQ)

Experimental conditions	Volume fraction, %				Number of Ki-67$^+$ cells per 1000 μ^2
	acini	stroma	acinar epithelium	acinar lumen	
Control	84 (79; 86)	16 (15; 21)	48 (45; 51)	34 (30; 38)	0.49 (0.40; 0.60)
Sulpiride 30 days					
day 10 after withdrawal	86 (82; 93)	14 (8; 18)	51.5 (50; 59)	36.5 (25; 41)	1.64 (1.34; 2.20)
					p=0.0006
day 30 after withdrawal	81 (77; 87)	19 (13; 23)	53 (51; 55)	30 (24; 31)	0.83 (0.57; 1.04)
					p=0.03
Sulpiride 60 days					
day 10 after withdrawal	91 (90; 95)	9 (5; 10)	61 (59; 67)	29 (27; 30)	1.85 (1.45; 2.53)
	p=0.003	p=0.001	p=0.03	p=0.02	p=0.00001
day 30 after withdrawal	89 (89; 90)	11 (10; 11)	71 (63; 72)	19 (17; 22)	0.96 (0.81; 1.24)
	p=0.06	p=0.04	p=0.02	p=0.002	p=0.02

Note. Significance of differences from the control.

creased volume fraction of acinar epithelium, narrowing of acinar lumens, and reduced volume fraction of the stroma. At the same time, serum level of prolactin remained unchanged, while PSA level increased.

BPH in humans is characterized by hyperplasia of acinar epithelium and stroma. However, the pathogenesis of PG hyperplasia is a complex pathological process and its mechanisms are poorly understood. A possible role in the development of BPH in humans can be played by inflammation. In patients with asymptomatic bacterial prostatitis, the content of T lymphocytes in PG is increased and some authors propose that BPH is

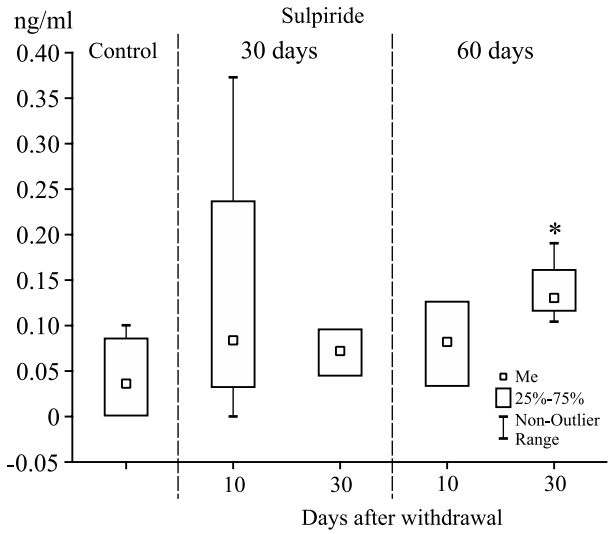

Fig. 1. Changes of PSA level in rat serum after the administration of sulpiride for 30 and 60 days and in 10 and 30 days after withdrawal. *p=0.02 in comparison with the control.

an immune-mediated inflammatory disease [12]. However, most researchers believe that BPH development is driven by age-related hormonal changes [2,8]. Clinical studies showed that BPH more rapidly develops in men with hormone-mediated metabolic disorders (insulin-resistance, abdominal obesity, hypertonia, hyperglycemia, and reduced HDL concentration) than in subjects with normal hormone levels. The role of androgens in BPH development is discussed for many years. The severity of androgen deficiency correlates with the incidence of BPH in humans, but it is not the only condition for the disease. BPH does not develop in males with low testosterone levels, for example, in subjects castrated before puberty or in subjects with hypopituitarism [5]. The estradiol-to-testosterone ratio increases with age, which also can be an important factor of BPH development.

In the experimental models of BPH used by us, no hyperplasia of acinar epithelium was detected by morphometric methods in ventral PG lobes in mature Sprague-Dawley rats after administration of sulpiride in dose of 40 mg/kg for 30 days and in 10 and 30 days after withdrawal, but the content of proliferating Ki-67$^+$ cells was elevated. After sulpiride administration for 60 days, the increase in the number of Ki-67$^+$ epithelial cells in the acini was associated with stable hyperplastic changes confirmed by morphometric studies and persisting in 10 and 30 days after sulpiride withdrawal. It should be noted that PSA concentration in the blood was elevated after administration of sulpiride for 60 days and in 30 days after its withdrawal.

Hyperplasia of PG is usually modeled in white outbred or Wistar rats. In our study, we used mature

TABLE 2. Changes of Prolactin Level in the Blood Serum after a Course Administration of Sulpiride (40 mg/kg) for 30 and 60 Days and in 10 and 30 days after Withdrawal (Me (LQ-UQ); ng/ml)

Experimental conditions	Administration for 30 days		Administration for 60 days	
	day 10 after withdrawal	day 30 after withdrawal	day 10 after withdrawal	day 30 after withdrawal
Control (intact animals)	8.96 (7.41-12.61)	10.92 (5.99-16.67)	10.53 (9.07-12.00)	14.36 (11.62-16.12)
Sulpiride	6.78 (3.16-8.30)	6.41 (4.93-8.30)	6.75 (3.88-8.35)	16.29 (6.00-29.88)

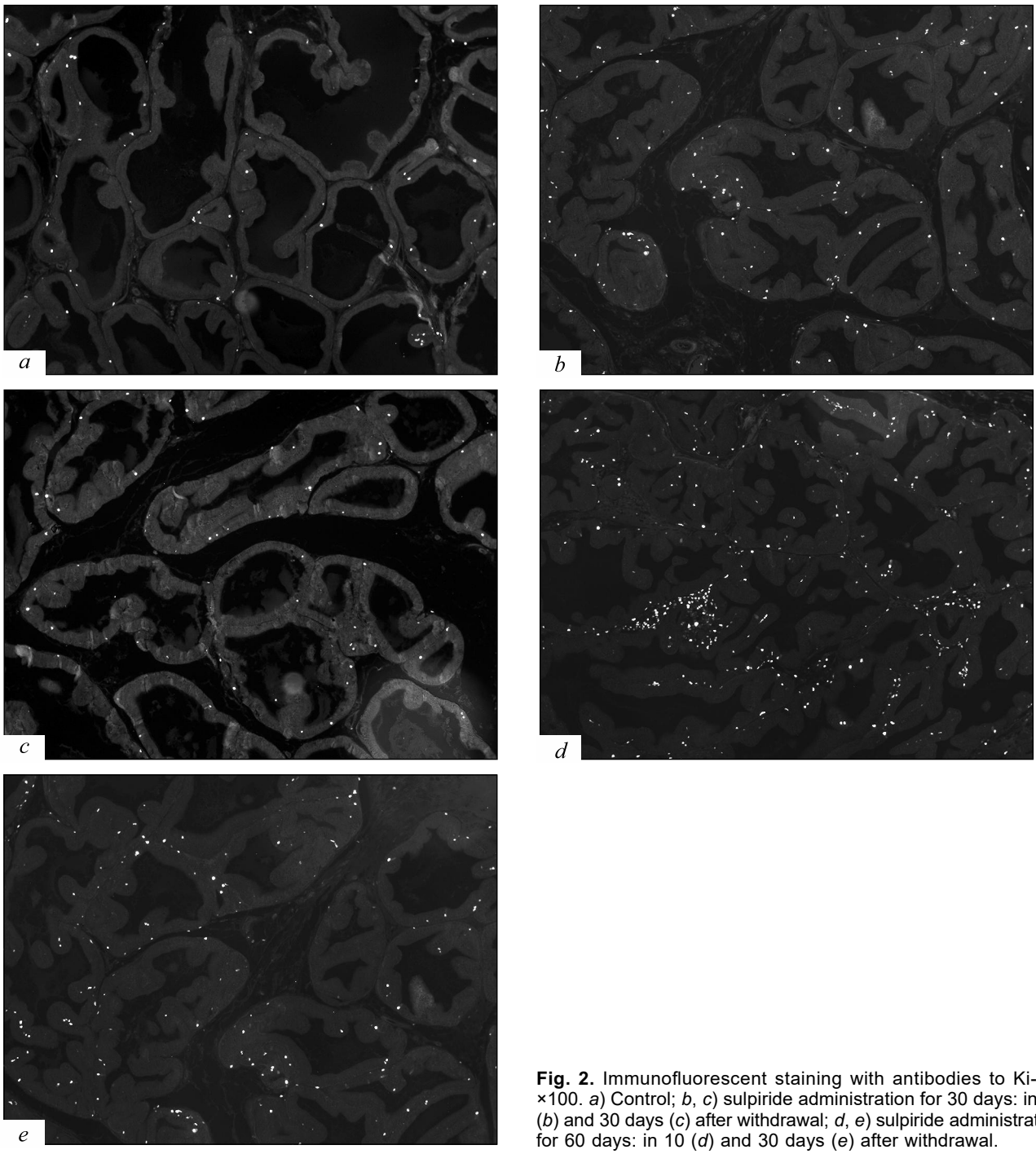

Fig. 2. Immunofluorescent staining with antibodies to Ki-67; ×100. *a*) Control; *b*, *c*) sulpiride administration for 30 days: in 10 (*b*) and 30 days (*c*) after withdrawal; *d*, *e*) sulpiride administration for 60 days: in 10 (*d*) and 30 days (*e*) after withdrawal.

Sprague-Dawley rats for BPH modeling, because these animals are characterized by low tolerance to stress and initially high sensitivity to hypoxia and, in comparison with Wistar rats, has more pronounced stroma in their parenchymatous organs [7]. Similar to study [1], we showed that sulpiride dose of 40 mg/kg is optimal for BPH modelling. We also studied possible reversibility of structural changes in PG at different terms after sulpiride withdrawal and showed that hyperplastic changes in PG induced by sulpiride administration in dose of 40 mg/kg for 60 days verified by morphometry and by counting of epithelial Ki-67$^+$ cells persisted for 10 and 30 days after sulpiride withdrawal. This should be taken into account in preclinical studies of drug efficiency.

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