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Prostatotropic Action of Glycyrrhizic Acid Disodium Salt in Benign Prostatic Hyperplasia Models

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The prostatotropic activity of glycyrrhizic acid disodium salt (Na_2GA) was studied in the models of benign prostatic hyperplasia (BPH) induced by chronic injection of sulpiride (40 mg/kg intraperitoneally for 8 weeks) or testosterone propionate (20 mg/kg subcutaneously for 4 weeks) in the Wistar rats. The oral administration of Na_2GA in a dose of 100 mg/kg produced a moderate antiproliferative effect in both BPH models resulting in reduced volume density of prostatic epithelium (in the testosterone model) and increased volume density of the glandular lumen (in both models). The observed prostatotropic effects of Na_2GA were similar to those of Permixon and finasteride, but they were less pronounced as confirmed by smaller drops in epithelial volume density and epithelial-to-stromal ratio compared to the effects of both reference drugs.

Key Words: *benign prostatic hyperplasia; glycyrrhizic acid; sulpiride; testosterone; morphology*

Glycyrrhizic acid is a commercial metabolite of *Glycyrrhiza* incorporating triterpenoid aglicon structurally similar to endogenous 11-ketosteroids. This structural feature explains synergism of a triterpenoid with glucocorticoids and a broad spectrum of the pharmacological effects exerted by *Glycyrrhiza* including the anti-inflammatory, anti-allergic, antiulcer, antioxidant, and antiproliferative ones [4]. The available data conclude that glycyrrhizic acid disodium salt (Na_2GA) affects steroidogenesis of sex hormones (testosterone included) via inhibition of 3β- and 17β-hydroxysteroid dehydrogenases and 17- and 20-dehydrolyases [5]. It was also shown that glycyrrhizin and Na_2GA could suppress production of testosterone in cultured testicular Leydig cells stimulated by luteinizing hormone [5].

The data on the effects of Na_2GA and licorice extract on testosterone level in healthy men are contradictory, but there are indications that these agents possess estrogenic and antiandrogenic properties [5]. The pro- and antiestrogenic activities were also found in other triterpenoids such as betulin and its derivatives, ursolic acid, celastrol, and cucurbitacin [9]. It is no wonder that these data attract acute attention to triterpenoids viewed as the modifiers of hormone-dependent processes including the benign and malignant proliferative diseases.

This work was designed to study possible prostatotropic effects of Na_2GA in the models of benign prostatic hyperplasia (BPH) in rats induced by repeated injections of sulpiride or testosterone.

MATERIALS AND METHODS

Experiments were carried out on pubertal male Wistar rats (n=40) weighing 320-450 g, which were obtained from Breeding Department of Federal Research Center Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences. The rats were maintained under vivarium conditions in accordance with recommendations of European Convention for

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Protection of Vertebrate Animals used for Experiments and Other Scientific Purposes (Strasbourg, 1986).

Two series of experiments were conducted in sulpiride (prolactin-dependent) and testosterone (androgen-dependent) BPH models. The prolactin-dependent BPH was induced by *intraperitoneally* injection of sulpiride (Eglonyl, Sanofi-Winthrop Industrie) in a dose of 40 mg/kg/day for 60 days [1,12]. The androgen-dependent BPH was provoked by intracutaneous injections of testosterone propionate (Dal'khimpharm) in a dose of 20 mg/kg/day for 28 days [10]. Na_2GA (Shaanxi Sciphar Biotechnology) was administered *per os* in water solution (100 mg/kg, [4]) 1 h prior to parenteral administration of the corresponding inductor. The reference drugs Permixon (Pierre Fabre Medicament) and finasteride (Penester, Zentiva) were administered *per oral* in the doses of 50 and 10 mg/kg, respectively [8,11]. The control rats received the equivalent amount of water, and the intact ones (used to assess the baseline parameters) were not exposed to any agents.

In each series, the rats were randomized into 4 equal groups (5 animals per group). Groups 1A and 1B comprised intact rats in both series. In water-treated control groups 2A and 2B, BPH was respectively provoked with sulpiride and testosterone propionate. The reference groups 3A and 3B comprised BPH rats treated with Permixon or finasteride. The experimental groups 4A and 4B consisted of BPH rats treated with Na_2GA.

The rats were sacrificed by instantaneous decapitation followed by excision of prostate gland (PG), which was fixed in 10% neutral formalin. The specimens were routinely processed in a MICROM (Carl Zeiss) histological system. The sections (4-5 μ) were stained with hematoxylin and eosin as well as with orange G—PAS—hematoxylin stain to visualize the structure of connective tissue. The histological specimens were examined under an AxioSkop 40 light microscope. The microphotos were obtained with an AxioCam MRc digital camera (0.63×) equipped with 10× or 20× objectives.

The morphometric analysis assessed the volume density (Vv) of glandular epithelium, glandular lumina, and stroma in several sites of PG dorsolateral surface, thereupon the epithelial-to-stromal ratio was calculated [3]. The data were analyzed statistically using Statistica 7.0 software (StatSoft, Inc.). Significance was assessed with parametrical Student's *t* test and non-parametrical Mann—Whitney *U*-test at $p<0.05$. The results are summarized as $m \pm SEM$.

RESULTS

Both models of BPH based on hormonal induction of androgens are most adequate to examine the prostatotropic properties of novel and promising agents acting by inhibition of 5α-reductase and consequential downregulation of dihydrotestosterone product in PG [6,7].

In both series of experiments with intact rats (groups 1A and 1B), the structure of dorsolateral portion of PG was typical. The secretory compartment of PG consisted of the end pieces and excretory ducts of numerous glandules of different size and location. PG glandules were predominantly lined with simple cuboidal or prismatic epithelium, as well as with stratified prismatic epithelium. There were no inflammatory or degenerative abnormalities in PG tissue of intact rats.

After a 60-day-long administration of sulpiride or 28-day-long injections of testosterone in the corresponding groups 2A and 2B, we observed the development of focal glandular hyperplasia. The foci of hyperplasia of glandular epithelium with formation of papillary structures were located in acinar lumina (Fig. 1, *a*, *b*). The glandular epithelium in the acini was tall prismatic with hyperchromatic nuclei (multilayered in some locations) and large number of mitoses, which had no signs of atypia. Hyperplasia of acinar structures was paralleled with disturbed outflow of the secret from the acini and their cystic dilation accompanied by epithelial atrophy (applanation). The glandular lumina were filled with eosinophilic secret. A moderate venous plethora and stromal edema were observed in all portions of PG. Importantly, epithelial hyperplasia and hemodynamic abnormalities were most pronounced in group 2B consisting of the rats with testosterone model of BPH. The glandular epithelium was characterized by the development of vacuolar dystrophy (Fig. 1, *b*). Thus, the revealed signs of acinar hyperplasia corresponded to hyperplasia stages III-IV [2].

In rats of the reference groups administered with Permixon (group 3A) or with finasteride (group 3B) against the background of sulpiride or testosterone, respectively, PG demonstrated diminished hyperplastic processes in the glandular epithelium (Fig. 1, *c*, *d*). The morphological pattern of detected alterations in all rats corresponded to stages I-II focal papillary hyperplasia. The acinar glandular epithelium was determined as prismatic without pronounced signs of atypia. Both groups retained the hemodynamic abnormalities such as venous plethora, edema, as well as infiltration of lymphocytes and macrophages into stroma. The signs of partial disturbance in outflow of secreted agents from acini remained together with cystic expansion and atrophy of the epithelium. The glandular lumina were filled with the secret looking like eosinophilic substance. In the glandular epithelium, the vacuolar dystrophy remained during administration of testosterone and finasteride.

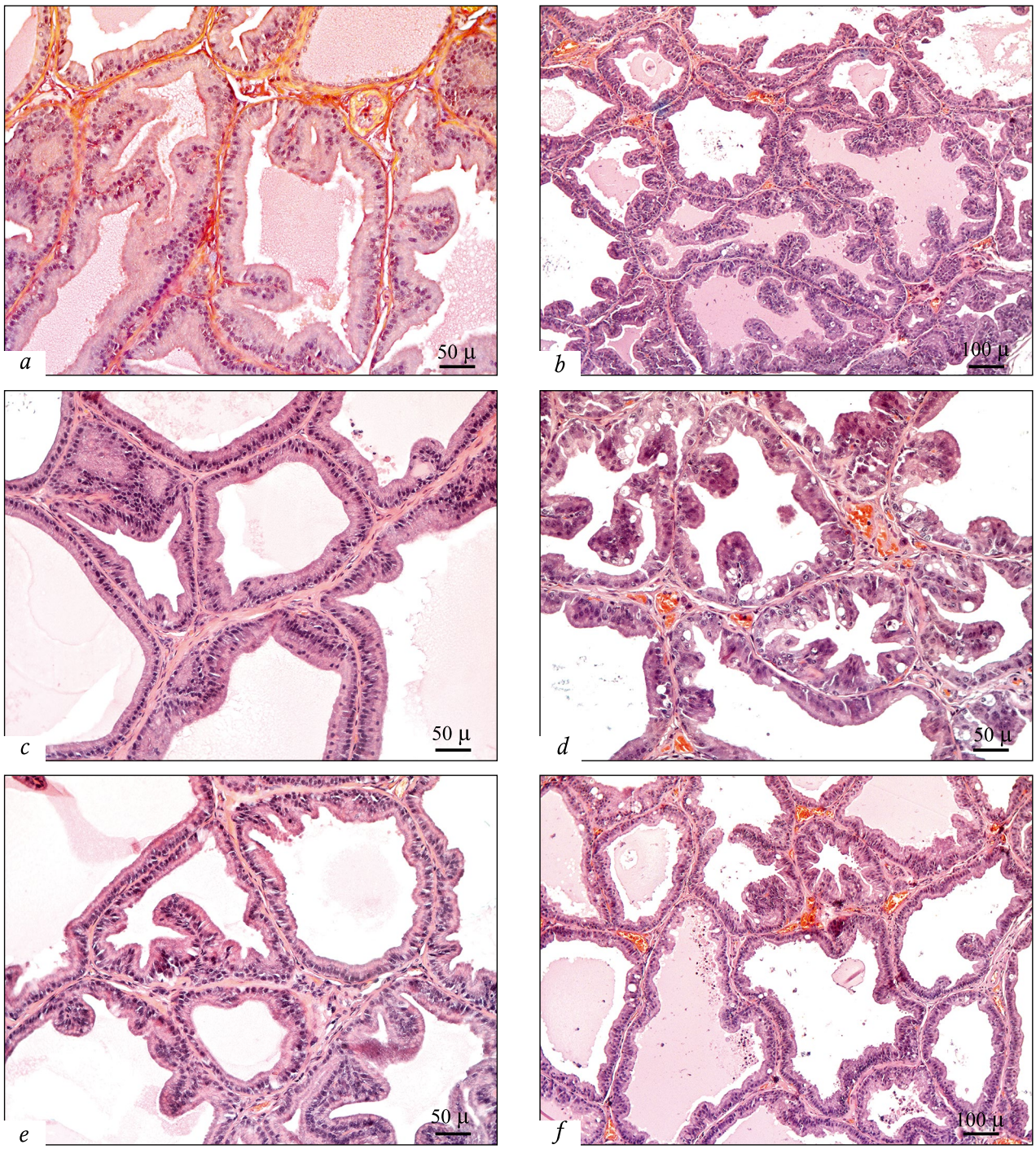

Fig. 1. The effect of Permixon, finasteride, and Na_2GA on PG morphology in sulpiride (*a*, *c*, *e*) and testosterone (*b*, *d*, *f*) BPH models. Staining with orange G—PAS—hematoxylin (*a*) or hematoxylin with eosin (*b*-*f*). *a*) Papillary form of BPH after a 60-day-long administration of sulpiride; *b*) pronounced venous plethora and vacuolar dystrophy in BPH provoked by 28-day-long injection of testosterone; *c*) moderation of hyperplastic processes in epithelium against the background administration of sulpiride and Permixon; *d*) moderation of hyperplastic processes in epithelium and maintenance of degenerative and hemodynamic alterations against the background administration of testosterone and finasteride; *e*) insignificant epithelial applanation against the background administration of sulpiride and Na_2GA; *f*) flattening of glandular epithelium and maintenance of vacuolar dystrophy and venous plethora against the background administration of testosterone and Na_2GA.

Administration of Na_2GA against the background of sulpiride (group 4A) or testosterone (group 4B) resulted in insignificant positive dynamics of pathologic process. In all rats of 4A and 4B groups (as in groups 3A and 3B administered with Permixon and finasteride), the study revealed the adenotic-type papillary

form of focal hyperplasia in the secretory portion of PG (Fig. 1, *e*, *f*). In acini, the glandular epithelium was high and prismatic with hyperchromatic nuclei. In all rats, there were desquamation of superficial epithelial layers into glandular lumina, cystic dilation of the glandules accompanied by atrophy of the epithelium, pronounced venous plethora, edema, and insignificant lymphomacrophagal infiltration into stroma. The periurethral area retained the pronounced hyperplastic epithelial alterations with formation of papillary projections. The fine fibers of loose fibrotic connective tissue were predominantly located near the acini. At this, administration of Na_2GA against the background of testosterone was accompanied by moderate inflammatory and degenerative processes including vacuolar dystrophy, epithelial atrophy, and epithelial desquamation into the glandular lumina (Fig. 1, *f*).

In groups A rats with sulpiride-induced BPH (modeled hyperprolactinemia), morphometry revealed the following. In experimental control group 2A rats "treated" with water, there were an increasing Vv trend of glandular epithelium (by 1.2 times) and decreasing Vv trend of glandular lumina (by 1.3 times) in comparison with intact control group 1A, but both trends were insignificant (Table 1). The treatment of groups 3A and 4A rats with the reference drug Permixon or experimental agent Na_2GA, respectively, demonstrated a decreasing Vv trend of epithelium and increasing Vv trend of glandular lumina in comparison with experimental control group 2A; at this, both parameters virtually did not differ from the intact control levels measured in group 1A rats. In Permixon-treated (group 3A) rats, the epithelial-to-stromal ratio was 3-fold smaller than that in water-treated experimental control group 2A ($p \leq 0.05$).

In groups 2A-4A rats, stromal Vv did not significantly differ from the corresponding parameter in intact control 1A rats. However, in rats treated with Permixon (group 3A), this parameter was higher by 1.4, 1.5, and 2 times in comparison with the respective values in groups 1A (intact control), 2A (experimental control), and 4A (experimental Na_2GA-treated rats); at this, the difference with 4A group was significant ($p \leq 0.05$). Probably, the effect of Permixon was exerted by its active component, β-sitosterol, which demonstrated not only the antiandrogenic activity by loosening the bond between dihydrotestosterone and androgen receptor [7], but as a phytoestrogen, it also modulated the activity of estrogenic receptors in PG stroma thereby stimulating its growth [9].

In groups B rats with testosterone model of BPH, the morphometric analysis revealed significant elevation of Vv of glandular epithelium in experimental control group 2B rats by 1.8 times ($p \leq 0.001$) relatively the respective parameter in intact control group 1B. In contrast, both reference drug finasteride (group 3B) or experimental agent Na_2GA (group 4B) significantly decreased Vv of glandular epithelium by 1.5 and 1.2

TABLE 1. Effect of Na_2GA on Volume Density (Vv) of Structural Elements of PG in Sulpiride Model of BPH in Rats (*m*±*SEM*)

Group	Vv			Epithelial-to-stromal ratio
	epithelium	stroma	glandular lumina	
1A (intact control)	0.35±0.03	0.14±0.01	0.50±0.05	2.48±0.11
2A (experimental control: BPH+H_2O)	0.43±0.06	0.13±0.03	0.37±0.05	3.21±0.76
3A (BPH+reference Permixon)	0.32±0.03^{+}	0.20±0.04	0.50±0.06	1.10±0.27^{**+}
4A (BPH+experimental Na_2GA)	0.37±0.04	0.10±0.02^{o}	0.52±0.05^{+}	3.07±0.69^{o}

Note. *$p \leq 0.05$, **$p \leq 0.01$ in comparison with group 1A; $^{+}p \leq 0.05$ in comparison with group 2A; $^{o}p \leq 0.05$ in comparison with group 3A.

TABLE 2. Effect of Na_2GA on Volume Density (Vv) of Structural Elements of PG in Testosterone Model of BPH in Rats (*m*±*SEM*)

Group	Vv			Epithelial-to-stromal ratio
	epithelium	stroma	glandular lumina	
1B (intact control)	0.34±0.03	0.20±0.02	0.45±0.04	1.78±0.30
2B (experimental control: BPH+H_2O)	0.62±0.02***	0.17±0.01	0.23±0.01***	3.86±0.42**
3B (BPH+reference finasteride)	0.41±0.03^{+++}	0.15±0.01*	0.44±0.03^{+++}	2.75±0.27*
4B (BPH+Na_2GA)	0.51±0.04^{**+}	0.18±0.03	0.31±0.03^{*o}	3.09±0.54*

Note. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ in comparison with group 1B; $^{+}p \leq 0.05$, $^{+++}p \leq 0.001$ in comparison with group 2B; $^{o}p \leq 0.05$ in comparison with group 3B.

times, respectively, in comparison with this parameter in experimental control group 2B (Table 2). While Vv of glandular epithelium did not significantly differ in intact control (1B) and reference drug (3B) groups, it was significantly greater by 1.5 times in rats treated with Na_2GA (group 4B) in comparison with intact control level ($p \leq 0.01$, Table 2).

Similar relationships were observed for Vv of glandular lumina: finasteride elevated this parameter 1.9-fold relatively the experimental control level (and made it equal to that in intact control group), whereas the treatment with Na_2GA was characterized by increasing trend of this parameter only by 1.3 times relatively to experimental control value (Table 2). In groups 2B-4B rats, the stromal Vv did not significantly differ from the corresponding parameter in intact control (group 1B) rats.

Analysis of volumetric proportions between glandular epithelium and stroma in the groups with sulpiride-provoked BPH revealed pronounced drops of epithelial-to-stromal ratio by 2.2 and 2.9 times induced by reference drug Permixon relatively intact control (1A) and experimental control (2A) levels, respectively (Table 1). In contrast, Na_2GA produced no significant effect on this parameter in rats with sulpiride-provoked BPH.

In rats with testosterone-provoked BPH, the epithelial-to-stromal ratio remained increased in comparison with intact control level established after the use of reference drug finasteride or experimental agent Na_2GA by corresponding 1.5 and 1.7 times ($p \leq 0.05$). However, this parameter demonstrated a decreasing trend in comparison with experimental control level by corresponding 1.4 and 1.2 times (Table 2).

Thus, the data obtained in both BPH models showed that intragastric administration of Na_2GA (100 mg/kg) induced similar but less pronounced prostatic alterations in comparison with those provoked by Permixon or finasteride. These alterations were manifested by diminished Vv of glandular epithelium and dilated glandular lumina in comparison with the experimental control rats. This inference was corroborated by comparison of epithelial-to-stromal ratios in the groups treated with Na_2GA or reference drugs.

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