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Morphological Assessment of Prostatotropic Activity of (3,5-Dimethyl-4-Hydroxy)Benzyl Thiododecane on a Model of Benign Prostatic Hyperplasia in Rats

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Prostatotropic activity of (3,5-dimethyl-4-hydroxy)benzyl thiododecane (T-DD) was evaluated on a model of benign prostatic hyperplasia induced in Wistar rats by chronic (2 months) intraperitoneal administration of sulpiride (40 mg/kg). Morphometric analysis of the dorsolateral lobe of the prostate showed that after the 2-month course of intragastric T-DD (100 mg/kg) administered in parallel with sulpiride, the volume density of glandular epithelium decreased by 1.7 times, while the volume density of prostate stroma increased by 2 times. After administration of the reference drug Permixon at a dose of 50 mg/kg, the volume densities of epithelium decreased by 1.3 times and stromal volume density increased by 1.5 times. The observed effects are presumably related to suppression of 5α-reductase activity and modulation of estrogen receptors in the prostate.

Key Words: *benign prostatic hyperplasia*; *sulpiride*; *benzyl thiododecane; Permixon; morphology*

According to modern views, one of the main causative factors of benign prostatic hyperplasia (BPH) is imbalance between estrogens and androgens appearing against the background age-related decrease in testicular testosterone [2,3,7]. This imbalance of sex hormones is accompanied by up-regulated secretion of prolactin [10], which markedly affects the epithelial-stromal interactions in the prostate gland (PG) and disturbs secretion of paracrine and autocrine factors regulating the growth, differentiation, and remodeling of PG [9,12]. An important role in these processes is played by compensatory increase in dihydrotestosterone synthesis in epithelial and stromal cells resulting from activation of 5α-reductase II. In light of this, the use of 5α-reductase inhibitors is viewed as the most effective method of drug correction of proliferative processes in BPH [3]. In contrast, to prevent or treat BPH symptomatically at its early stages, low-toxic phytopreparations (most popular in USA and Europe) are used [11].

To this end, the patients are most frequently treated with preparations based on the extracts from *Serenoa repens* (Permixon, Prostamol-Uno) producing antiandrogenic, anti-inflammatory, anti-edematous, and antiproliferative effects and selectively affecting PG structure and function [7,11]. Permixon contains long-chain fatty acids (lauric, linoleic, linolenic, and myristic) and some phytosterols including β-sitosterol, campesterol, and stigmasterol exhibiting antihypoxic and antifibrotic activities [8]. Limited choice of such phytopreparations and the hope to enhance their specific activity motivate the researchers to search for novel prostatic protectors of plant or synthetic origin. Of them, a promising agent is phenolic antioxidant (3,5-dimethyl-4-hydroxy)benzyl thiododecane (T-DD) synthesized at the Novosibirsk State Pedagogical Uni-

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versity [6]. It was previously demonstrated that this compound exhibits high antioxidant activity, produces membrane stabilizing, antiproliferative, cytoprotective, and anti-ischemic effects, and improves hemorheological parameters in various tissues [4,5].

This work was designed to assess prostatotropic activity of T-DD in Wistar rats with experimental BPH provoked by long-term administration of sulpiride.

MATERIALS AND METHODS

The experiments were carried out on mature male Wistar rats weighing 320-450 g obtained from the vivarium of Federal Research Center Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences. All procedures were carried out in strict adherence to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The rats were randomized into 4 groups of 5 animals each. Group 1 comprised intact rats. Animals of experimental groups 2, 3, and 4, received intraperitoneal injection of sulpiride (Eglonyl, Sanofi, 40 mg/kg) over 2 months for modeling BPH [1,15]. In parallel, the animals orally received water (group 2, control), Permixon (Pierre Fabre Medicament) in a dose of 50 mg/kg in water suspension [13] (group 3), or T-DD in a dose of 100 mg/kg in refined sunflower oil (group 4). The solvents and dose of T-DD were chosen based on its lipophilicity and previous data on its biological activity [5].

In two months after the start of the experiment, all rats were decapitated. PG was isolated and fixed in 10% neutral formalin. The specimens were processed routinely with a MICROM histological tissue processor (Carl Zeiss). The tissue sections (4-5 μ) were stained with hematoxylin and eosin, after van Gieson to reveal connective tissue structures, and complex PAS—hematoxylin—orange G staining was carried out. The sections were examined under an AxioSkop40 light microscope. The microphotographs were obtained with an AxioCam MRc camera at ×200.

Morphometry was carried out in several areas of the dorsolateral part of PG. Volume densities of the glandular epithelium, lumens of glandules, and stroma were determined and the epithelium/stroma volume ratio was calculated [3].

The data were analyzed statistically using Statistica 7.0 (StatSoft, Inc.) software. Significance of differences was evaluated by parametric Student's *t* test and non-parametric Mann—Whitney *U* test at $p<0.05$. The results are presented as *m*±*SE*.

RESULTS

Hyperprolactinemia induced by long-term administration of sulpiride provokes stromal and epithelial hyperplasia predominantly in PG lateral lobe often accompanied by inflammation [15]. In our study, the dorsolateral lobe of PG in intact rats had typical structure. The secretory area of this lobe was presented by terminal parts and excretory ducts of numerous glandules that differed in size and location. They were predominantly lined with single-layered cuboidal or prismatic epithelium as well as a multilayered prismatic epithelium (Fig. 1, *a*). No inflammatory or degenerative changes were found in PG of intact rats.

After a 2-month sulpiride administration, the development of focal glandular hyperplasia in PG of group 2 rats (experimental control) was observed (Fig. 1, *b*). The formation of additional satellite proliferative foci led to the appearance of papillary structures in the acinar lumen. Enhanced proliferative activity of acinar structures was paralleled by disturbances in secreta outflow from PG acini, resulting in their cystous dilatation. In acini, the glandular epithelium actively proliferated; it was presented by tall prismatic cells with hyperchromic nuclei, somewhere had multilayered structure with high number of mitoses, no signs of atypia were seen. In all PG zones, moderate venous plethora, edema, and collagenation of the stroma were observed. In the central zone, most glandules were lined with simple prismatic epithelium. Some glandules were lined with cuboidal epithelium and

TABLE 1. Effect of T-DD (100 mg/kg) and Reference Drug Permixon (50 mg/kg) on Volume Density of PG Structural Components in Wistar Rats with Sulpiride-Induced BPH (*m*±*SE*)

Group	Volume density, %			Epithelial/stromal volume ratio, %
	epithelium	stroma	lumens of glandules	
Intact rats (group 1)	0.35±0.03	0.14±0.01	0.50±0.05	2.48±0.11
Control (group 2)	0.43±0.06	0.13±0.03	0.37±0.05	3.21±0.76
Permixon (group 3)	0.32±0.03^{+}	0.20±0.04	0.50±0.06	1.10±0.27^{+}
T-DD (group 4)	0.25±0.04^{+}	0.27±0.01^{+}	0.44±0.01	0.98±0.35^{*+}

Note. $^{*}p<0.05$ in comparison with *intact rats, $^{+}$control.

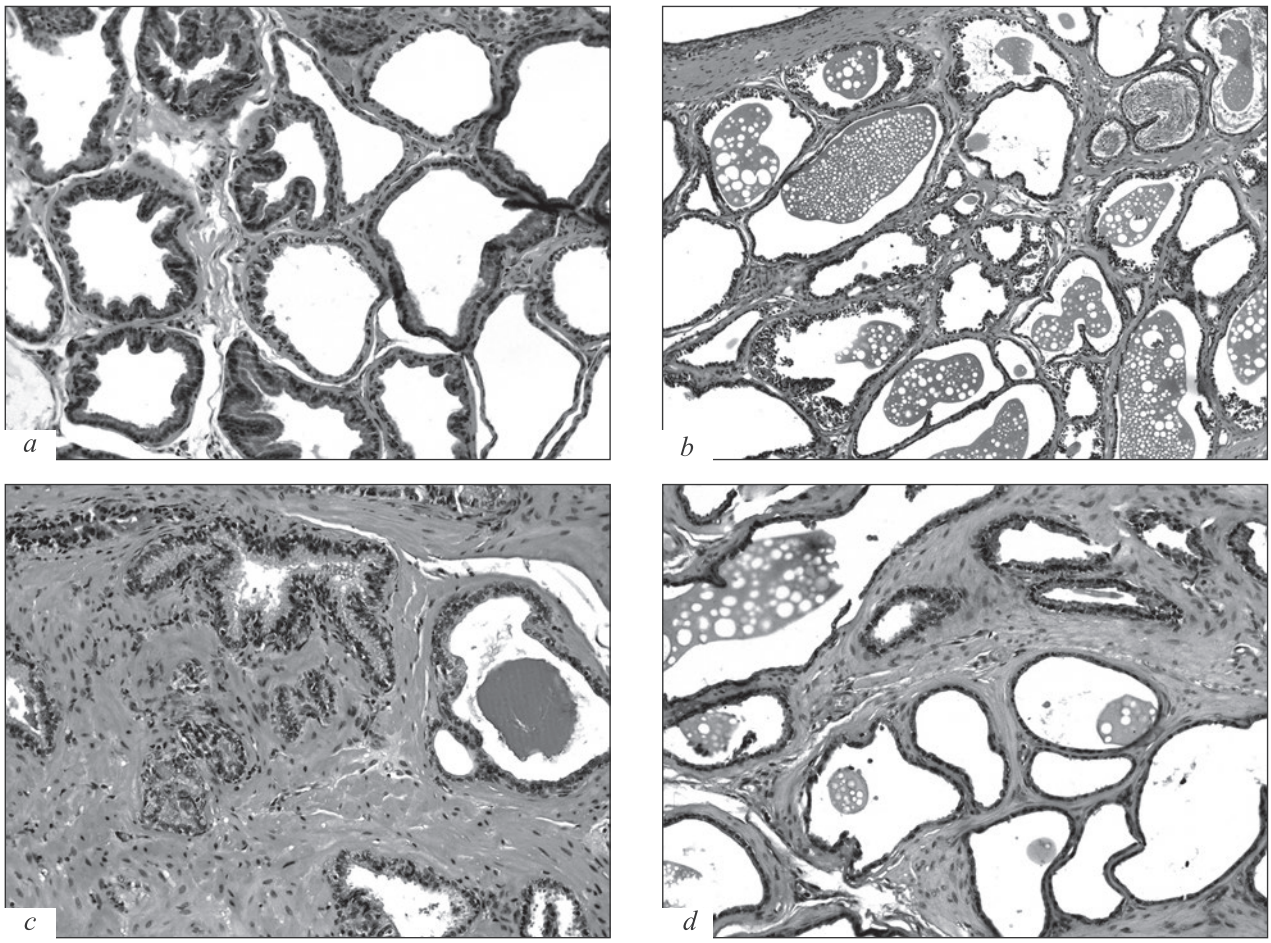

Fig. 1. Morphological effects of T-DD and reference drug Permixon in PG of Wistar rats with modeled BPH provoked by 2-month-long injections of sulpiride. The sections were stained with hematoxylin and eosin (*a*-*c*) or PAS—hematoxylin—orange G (*d*), ×200. *a*) PG of intact (group 1) rat; *b*) papillary form of BPH in central zone of PG in a control (group 2) rat; *c*) proliferation of glandular epithelium in a group 3 rat treated with Permixon (50 mg/kg); high prismatic epithelium with no signs of atypia; *d*) reduced height of glandular epithelium and reduced number of mitoses in a group 4 rat treated with T-DD (100 mg/kg).

cystously dilated. The glandular lumens were filled with eosinophilic secreta. Overall, the revealed signs of proliferation attested to hyperplasia stages III-IV.

In PG of group 3 rats treated with the reference drug Permixon, the hyperplastic processes were less pronounced than in group 2 rats. In all group 3 rats, PG hyperplasia attested to stages I-II of papillary focal hyperplasia. The glandular epithelium in the acini was prismatic without pronounced signs of proliferation or atypia (Fig. 1, *c*). In addition, the number of mitotic epitheliocytes was lower than that in group 2 rats. At this, the glandular lumens somewhat reduced and the stroma was enlarged.

In group 4 rats (treated with T-DD), more pronounced correction of hyperplastic processes was revealed in comparison with group 3 rats treated with Permixon. In the former, the structure of glandular epithelium was similar to that in intact rats. It had no salient proliferation signs; the glandular epithelium had only single papillary enlargements (Fig. 1, *d*). PG stroma was infiltrated with single mast cells and macrophages reflecting stimulation of metabolic processes. Van Gieson staining revealed moderately pronounced collagenation of periacinar structures.

Morphometry of structural elements of PG revealed significant ($p<0.05$ in comparison with control rats) decrease in volume density of glandular epithelium in groups 3 and 4 rats by 1.3 and 1.7 times, respectively (Table 1). In these experimental groups, the volume densities of glandular lumens did not significantly differ from those in intact rats. In groups 3 and 4, the volume density of PG stroma increased, by 1.5 and 2 times, respectively, in comparison with the control or intact rats. In comparison with intact rats, the stromal volume density was not elevated in control rats, and only changes in volume parameters of the secretory compartment of PG were found. For instance, volume density of the epithelium in control rats was elevated (by 23%) and volume density of glandular lumens was reduced (by 26%, Table 1). Opposite changes in epi-

thelial and stromal volume densities in groups 3 and 4 rats resulted in pronounced decrease of epithelium/stroma volume ratio in comparison with intact (by 2.3 and 2.5 times, respectively) or control (by 2.9 and 3.3 times, respectively) rats ($p \leq 0.05$).

It is known that hyperprolactinemia increased the expression of 5α-reductase in PG resulting in dihydrotestosterone elevation and enhanced cell proliferation in PG [10,15]. It was also shown that sulpiride increases the content of mRNA of both 5α-reductase isoforms and stimulates the expression of Bcl-2 anti-apoptotic proteins in rats [14]. Hence, it can be hypothesized that the observed decrease in the volume density of glandular epithelium induced by Permixon or T-DD is related to inhibition of 5α-reductase. A similar mechanism was described for Permixon [8]. Here, the observed enlargement of the stromal component in the groups treated with Permixon and T-DD can be determined by the interaction of the test agents with estrogen receptors in PG. Notably, numerous natural phytoestrogens (oxysterols, terpenes, polyphenols, flavonoids, and isoflavonoids) as well as synthetic phenols such as bisphenol A are modulators of these receptors [12]. Activation of α-estrogen receptors is associated with hyperplasia and stromal inflammation, whereas activity of β-estrogen receptors is related to the antiproliferative effect in the glandular epithelium [11,12]. Thus, significant decrease in the epithelium/stroma volume ratio (Table 1) can be viewed as a corroborative argument for this hypothesis. Additionally, the possibility of elevation of endogenous estrogens due to the action of T-DD or Permixon on aromatase expression cannot be excluded [12].

Thus, in modeled BPH provoked by a long-term administration of intraperitoneal sulpiride, T-DD (100 mg/kg) induced more pronounced, in comparison with Permixon (50 mg/kg), inhibition of hyperplasia of glandular epithelium and elevation of stromal volume density. Treatment with Permixon or T-DD significantly decreased the epithelium/stroma volume ratio in comparison with intact or control rats. Importantly, T-DD decreased this ratio more pronouncedly than Permixon, which highlights this agent as a promising launch pad to develop the novel remedies.

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