Oral exposure of sulpiride promotes the proliferation of Brown-Norway rat prostates

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Abstract. The aim of the present study was to establish an animal model of prostatic hyperplasia to explore the mechanisms of this disease. Sulpiride, a specific type 2 dopamine receptor antagonist, causes prostate toxicity by stimulating prolactin (PRL) production. Male Brown-Norway (BN) rats were treated intragastrically (i.g.) with sulpiride (40 and 120 mg/kg daily) and vehicle (i.g., daily) for 4 weeks. The results demonstrated that sulpiride-treatment resulted in increased prostate size, prostate lobe weight, epithelial height and acinar luminal area. Furthermore, prostate lobe weight, epithelial height and acinar luminal area of lateral lobes (LP) significantly increased. These effects were dose dependent. Sulpiride treatment increased serum PRL, follicle-stimulating hormone and testosterone levels, while serum luteinizing hormone levels were reduced. Immunohistochemical analysis revealed that proliferating cell nuclear antigen and B-cell lymphoma-2 were significantly increased in certain sulpiride treated groups. Furthermore, estrogen receptor (ER)-α and androgen receptors were upregulated, while ERβ was downregulated in LP. The expression of stromal cell biomarkers, including vimentin, fibronectin and α-smooth muscle actin were significantly increased in LP following 40 mg/kg sulpiride administration. These results suggest that sulpiride causes LP hyperplasia in BN rats by promoting proliferation and inhibiting prostate cell apoptosis via ERα and AR signaling.

Introduction

Benign prostatic hyperplasia (BPH) is a hyper-proliferative disease that reduces the quality of life of elderly men. The worldwide incidence of BPH is 20% in men at age 40, which rises to 70% by age 60 and 90% by age 90 (1,2). BPH is characterized by a four-fold increase of stromal cells, which results in prostate gland expansion; therefore, it is generally considered a proliferative stromal disease (3,4). BPH increases prostate size and tightens the urethra, producing symptoms in the lower urinary tract, including urinary intermittency, nocturia, frequency, dysuria, weak stream, incomplete emptying and suprapubic pain (5).

The pressures of modern society have led to the increased prevalence of mental health issues, which has prompted the production and use of various antipsychotic drugs (6). Antipsychotic drugs may cause toxic adverse effects, including menstrual disorder, amenorrhea, dysuria and constipation in the human reproductive system, particularly in the prostate (7), which has received increasing attention from clinicians.

Sulpiride, a specific type 2 dopamine receptor antagonist, produces various side effects, including insomnia, fatigue, tachycardia, liver dysfunction and delayed dyskinesia when the dose of sulpiride reaches 600 mg/day (8-11). PRL is associated with the growth and development of BPH (9,12,13). PRL levels increase, while testosterone (T) levels decrease with age, which indicates that PRL serves a key role in BPH development in the elderly (8,14). Ahonen *et al* (15) reported that PRL serves a primary role in the differentiation and proliferation of the prostate in rats and humans. The effects of PRL are mediated via signal transduction pathways triggered by PRL receptors (16). Słuczanowska-Głąbowska *et al* (9) revealed that PRL increases while T decreases in experimental rats that received metoclopramide. Morphological abnormalities were also observed in columnar epithelial cells of the lateral, dorsal and ventral lobes; however, prostate lobes did not exhibit morphological changes under hyperprolactinemia (7). Previous studies have indicated that PRL may promote prostate growth and cell proliferation synergistically with androgens (8,17) Conversely, it has also been reported that PRL acts independently of T in prostate growth (18). Additional studies have revealed that PRL stimulates the secretion of prostate proteins and conversion of T to dihydrotestosterone (19,20). The prostate is dependent on androgens, which serve a role in BPH (21). Furthermore, testosterone regulation, prostate structure and prostate function are influenced by tissue growth and the hypothalamus-pituitary-gonadal

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axis (22). T secretion is regulated by luteinizing hormone (LH) and follicle stimulating hormone (FSH) (23-27). Therefore, PRL may serve an important role in the regulation of prostate cell growth and differentiation.

Proliferating cell nuclear antigen (PCNA) mediates the proliferation of prostate cells in rats and, as such, is used as a marker of proliferation (28). In addition, B-cell lymphoma-2 (Bcl-2) is an anti-apoptotic protein that promotes prostate hyperplasia (29). Shi *et al* (30) revealed that estrogens contributed to the pathogenesis of BPH in elderly men. Estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) are expressed and are antagonistic; ERα mediates cell proliferation while ERβ regulates apoptosis (31,32). Furthermore, the androgen receptor (AR) was demonstrated to promote prostate cells proliferation in BPH development (33). Similar to ERs, various co-regulators interact directly with AR, enhancing or reducing its transcriptional activity (34).

In BPH, the relative ratio of stroma: Epithelium increases with disease progression (35). Fibroblasts, myofibroblasts and smooth muscle cells are the primary stromal components of prostate tissue (36). It has also been revealed that mesenchymal cell markers, including vimentin, fiber binding proteins (fibronectin) and smooth muscle actin-α (α-SMA) serve roles in BPH progression (37-39).

Studies that assess the mechanism of BPH primarily utilize rodents, including Sprague-Dawley rats (40), Wistar rats (41), as well as non-rodent animal models, including Beagle dogs (12). However, Brown-Norway (BN) rats are rarely employed to model BPH. Although *in vitro*/*in vivo* prostate models have been developed to explore the mechanism of benign hyperplasia, to the best of our knowledge, estrogen receptor subtypes, ARs and mesenchymal cell biomarkers (including vimentin, fibronectin and α-SMA) have not been assessed in the BN rat model. The aim of the present study was to establish a useful model of BPH and explore the mechanism of sulpiride-induced benign hyperplasia in male BN rats.

Materials and methods

Animals and housing. A total of 36 male BN rats (10 weeks old; 280±20 g) were obtained from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., (Beijing, China) and housed in standard polypropylene cages with sawdust bedding. Drinking water and a pellet diet (Shanghai Shilin Biological Technology Co., Ltd., Shanghai, China) were available *ad libitum*. Rooms were maintained at 20-26˚C with 40-70% humidity and a 12 h light/dark cycle. The present study was approved by the Shanghai Institute of Planned Parenthood Research Animal Care (Shanghai, China).

Animals were divided into three groups (n=12) according to body weight following 5 days acclimatization as follows: Vehicle group (290.0±49.0 g), 40 mg/kg sulpiride group (292.3±55.4 g) and 120 mg/kg sulpiride group (294.3±50.5 g). All animal procedures were approved by the Animal Care and Use Committee of Shanghai Institute of Planned Parenthood Research (Shanghai, China) and performed according to the Guide for the Care and Use of Laboratory Animals.

Reagents. Sulpiride (cat. no. SLBG4648V; purity, 100%) was obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Sodium carboxymethyl cellulose (CMC-Na; cat. no. 20140520; purity, 100%) was purchased from Sinopharm Group Co., Ltd. (Beijing, China). Sulpiride was dissolved in 0.5% CMC-Na. The characteristics of primary antibodies are presented in Table I. UltraSensitive™ SP (Mouse/Rabbit) IHC kit (cat. no. KIT-9710) and PBS were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd. (Fuzhou, China).

Experimental groups. Rats were randomly divided into three groups (n=12) according to body weight following acclimatization, treated daily with sulpiride (40 and 120 mg/kg, intragastrically) or vehicle (0.5% CMC-Na) for 4 weeks and weighed once per week as previously described (42). The 40 and 120 mg/kg dosages represent the therapeutic low and high dose used in human treatment (43,44). On day 29 (24 h following final treatment), 3% pentobarbital sodium (Sigma-Aldrich; Merck KGaA; cat no. P3761; 39 mg/kg) anesthesia was administered via intravenous injection and whole blood samples (~6-8 ml) were obtained from aorta-ventralis. Following euthanasia, a median abdominal incision was performed to expose the bladder and prostate. Prostates were harvested and divided into three sections, including ventral, dorsal and lateral lobes (VP, DP and LP, respectively) according to their position relative to the urinary bladder. The lobes were weighed and fixed in neutral 10% formalin for 24 h at room temperature.

Histopathology. VP, DP and LP tissues were embedded in paraffin, sectioned at 3 μm and submitted to routine hematoxylin for 5 min and eosin for 1 min (H&E) staining at room temperature. Histological changes were observed under an optical microscope at magnification, x40 for the acinar luminal area and magnification, x400 for the height of the prostatic epithelium (Nikon Eclipse 50i; Nikon Corporation, Tokyo, Japan). The height of the prostatic epithelium (HPE) and acinar luminal area were (ALA) assessed using Nikon NIS-Elements BR 3.1 software (Nikon Corporation). A total of 20 epithelial samples per rat (240 per group) were randomly selected for analysis by a blinded investigator.

Hormone level detection. Blood samples were harvested and centrifuged for 15 min (2,000 x g, 4˚C) to collect serum, which was immediately stored at -80˚C. Serum PRL (cat. no. DEV9966), FSH (cat. no. LS-F6305), T (cat. no. 582701) and LH (cat. no. 12281601A) levels were determined using specific ELISA assay kits obtained from Demeditec Diagnostics GmbH (Kiel, Germany), LifeSpan BioSciences, Inc. (Seattle, WA, USA), Cayman Chemical Company (Ann Arbor, MI, USA) and ENZO Life Sciences, Inc. (Farmingdale, NY, USA) according to the manufacturers' protocol. The absorbance was read using a microplate reader at 450 nm (Zenyth 200st; Biochrom Ltd., Cambridge, UK).

Immunohistochemical staining (IHC). Representative blocks of paraffin-embedded prostate tissues were fixed as above and sliced to a 4 μm thickness, dewaxed and rehydrated in a descending alcohol series. Sections were heated to 95-100˚C in a microwave for 20 min for antigen retrieval and washed with a 0.01 M sodium citrate buffer (pH 6.0). The UltraSensitive™

Table I. Characteristics of primary antibodies.

Primary antibodies	Supplier	Host species	Dilution	Retrieval	Incubation	Cat. no.
PCNA	Santa Cruz Biotechnology, Inc.	Rabbit	1:100	10 min x2	1 h; room temperature	Sc-7907
Bcl-2	Santa Cruz Biotechnology, Inc.	Rabbit	1:100	10 min x2	Overnight; 4˚C	Sc-492
ERα	Santa Cruz Biotechnology, Inc.	Rabbit	1:75	10 min x2	Overnight; 4˚C	Sc-7207
ERβ	ProteinTech Group, Inc.	Rabbit	1:100	10 min x2	Overnight; 4˚C	14007-1-AP
AR	Santa Cruz Biotechnology, Inc.	Mouse	1:100	10 min x2	Overnight; 4˚C	Sc-7305
Vimentin	BD Biosciences	Mouse	1:100	10 min x2	Overnight; 4˚C	550513
Fibronectin	BD Biosciences	Mouse	1:250	10 min x2	Overnight; 4˚C	610078
α-SMA	Santa Cruz Biotechnology, Inc.	Mouse	1:100	10 min x2	Overnight; 4˚C	Sc-53142

PCNA, proliferating cell nuclear antigen; Bcl-2, B-cell lymphoma-2; ERα, estrogen receptor-α; ERβ, estrogen receptor-β; AR, androgen receptor; α-SMA, α-smooth muscle actin; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; ProteinTech Group, Inc., Chicago, IL, USA; BD Biosciences, Franklin Lakes, NJ, USA.

SP (Mouse/Rabbit) IHC kit was used for peroxidase staining. Endogenous peroxidase was quenched with oxidase blocking solution (Reagent A) for 10 min at room temperature. Following blocking with normal non-immune serum (Reagent B) for 10 min at room temperature, sections were incubated with primary antibodies as presented in Table I. Primary antibodies were replaced with PBS in negative controls. The corresponding secondary antibodies (Reagent C) and Streptomyces antibiotic peroxidase solution (Reagent D) were added successively at room temperature for 10 min, followed by staining with 3,3'-diaminobenzidine for 3-10 min at 25˚C). Sections were then treated with hematoxylin at room temperature for 2 min, dehydrated in a descending series of alcohol, washed with xylene, mounted and observed under an optical microscope (magnification, x400; Nikon Eclipse 50i; Nikon Corporation) by a blinded investigator. Finally, mean optical densities were obtained using Image Pro-Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical analysis. Data were statistically analyzed using SPSS version 11.0 (SPSS, Inc., Chicago, IL, USA) and expressed as the mean ± standard deviation. Statistical comparisons were performed using one-way analysis of variance followed by Tukey's post hoc multiple comparison test. If statistically significant, differences between control and treatment groups were assessed using a least-squares means test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Body and prostate lobe weights. During the 4 weeks of sulpiride administration, animal body weights increased slightly compared with controls, however no significant differences were observed (Table II). Following treatment with 40 and 120 mg/kg/day sulpiride, prostate weight and relative prostate weight increased in a dose-dependent manner (Table II). In addition, treatment with sulpiride resulted in a dose-dependent increase of DP and LP wet and relative weights (Table III). In particular, LP in sulpiride groups was significantly increased ($P<0.05$ for sulpiride 40 mg/kg and $P<0.01$ for sulpiride 120 mg/kg). VP and relative weights in the 40 mg/kg sulpiride group were reduced compared with the control values, while sulpiride 120 mg/kg demonstrated higher values (Table III).

Histology. Histologically, proliferative features were more prominent in the sulpiride groups compared with the control group (Fig. 1). H&E staining revealed glandular prostatic hyperplasia with increased HPE and ALA in VP and LP of 40 and 120 mg/kg sulpiride groups (Figs. 1 and 2). In the sulpiride groups, the HPE of LP was significantly increased compared with control values ($P<0.01$; Table IV). Following 4 weeks of treatment with sulpiride, HPE was significantly increased in VP and LP tissues in the sulpiride groups compared with the control group ($P<0.01$; Table IV), particularly in the 120 mg/kg sulpiride group.

In the sulpiride groups, the ALAs of lobes were significantly increased compared with the controls, particularly in LP tissues ($P<0.01$; Table V; Fig. 3). Following 4 weeks of treatment with sulpiride, ALA in LP tissues was significantly increased compared with control values ($P<0.01$; Table V), particularly in the 120 mg/kg sulpiride (Table V; Fig. 3).

Serum PRL, FSH, T and LH levels. All groups treated with sulpiride exhibited higher PRL levels compared with the control group, particularly following treatment with 120 mg/kg sulpiride ($P<0.001$; Table VI). Furthermore, PRL levels demonstrated a dose-dependent increase. All groups treated with sulpiride had significantly increased T levels compared with the control group ($P<0.01$ for sulpiride 120 mg/kg and $P<0.001$ for sulpiride 40 mg/kg). Compared with the control group, the LH levels were significantly decreased in the sulpiride groups ($P<0.001$; Table VI). Additionally, the results determined that LH levels decreased as the sulpiride dose increased. FSH levels were increased in the sulpiride groups compared with controls, particularly in the 40 mg/kg sulpiride group ($P<0.001$; Table VI).

PCNA, Bcl-2, ERα, ERβ, AR, vimentin, fibronectin and α-SMA expression levels in prostate lobes. The expression levels of PCNA, Bcl-2, ERα, ERβ, AR and mesenchymal cell markers (including vimentin, fibronectin and α-SMA)

Table II. Effects of Sulpiride on body and prostate weight of benign hyperplasia prostate modeled Brown-Norway rats.

	Body weight (g)			Prostate	
Group	Initial	Final	Weight gain	Weight (g)	Relative weight (/100)
Control	290.0±49.0	305.9±39.1	15.0±37.1	0.567±0.140	0.185±0.034
Sulpiride (40 mg/kg)	292.3±55.4	312.5±41.5	20.0±45.3	0.637±0.095	0.206±0.033
Sulpiride (120 mg/kg)	294.3±50.5	312.9±42.8	18.6±44.7	0.784±0.200[a]	0.248±0.036[a]

Relative weight=(organ weight/terminal body weight) x100. Data are presented as the mean ± standard deviation (n=12). [a]P<0.05 vs. control.

Table III. Effects of sulpiride on prostate lobe weight of benign hyperplasia prostate modeled Brown-Norway rats.

	VP		DP		LP	
Experimental group	Weight (g)	Relative weight (/1,000)	Weight (g)	Relative weight (/1,000)	Weight (g)	Relative weight (/1,000)
Control	0.361±0.087	1.170±0.190	0.110±0.053	0.350±0.150	0.096±0.034	0.320±0.140
Sulpiride (40 mg/kg)	0.347±0.061	1.128±0.234	0.148±0.054[a]	0.472±0.167[a]	0.142±0.022[a]	0.459±0.076[a]
Sulpiride (120 mg/kg)	0.419±0.083	1.334±0.148	0.192±0.088[b]	0.595±0.221[b]	0.173±0.068[b]	0.549±0.171[b]

Relative weight=(organ weight/terminal body weight) x1,000. Data were presented as the mean ± standard deviation (n=12). [a]P<0.05 and [b]P<0.01 vs. controls. VP, ventral prostate; DP, dorsal prostate; LP, lateral prostate.

were immunohistochemically analyzed to further assess the sulpiride-induced signaling pathway in BPH. The results demonstrated that PCNA was primarily expressed in the nucleus of epithelial cells (Fig. 4A). Compared with the control group, sulpiride treatment resulted in increased PCNA levels in LP (P<0.01; Fig. 4C); however, no significant differences were observed in VP and DP. The Bcl-2 protein was primarily expressed in the cytoplasm and was significantly increased in VP, DP and LP tissues in the sulpiride groups compared with the controls (P<0.05 or P<0.01; Fig. 4).

The results of IHC demonstrated that ERα was primarily distributed in the epithelial cell nucleus (Fig. 5A) and ERβ was expressed abundantly in the basal epithelial layer within cell nuclei (Fig. 5B). In the 120 mg/kg sulpiride group, ERα levels in LP were slightly increased compared with control values (P<0.05; Fig. 5D). Similar results were observed in the 40 mg/kg sulpiride group (P<0.01; Fig. 5D). AR was expressed in the nucleus and cytoplasm of epithelial cells, but remained predominantly nuclear. The expression of AR was similar to that of ERα, unlike ERβ (Fig. 5D-F). Sulpiride 40 mg/kg significantly upregulated ERα and AR levels in LP (P<0.01) whilst sulpiride 120 mg/kg downregulated ERβ, ERβ was significantly increased in LP tissues treated with sulpiride 40 mg/kg (P<0.05; Fig. 5E). The effects of sulpiride treatment at 40 mg/kg were markedly more pronounced than those exhibited at 120 mg/kg.

Vimentin constitutes a specific protein marker of fibroblast cells (45) and was primarily expressed in mesenchymal cells (Fig. 6). Compared with the control group, treatment with 40 and 120 mg/kg sulpiride significantly upregulated vimentin in LP tissues (P<0.01; Fig. 6D); however no significant differences in vimentin levels were detected in VP and DP tissues. Fibronectin was primarily expressed in mesenchymal cells (Fig. 6B). Compared with controls, fibronectin expression in DP and LP following treatment with 40 mg/kg sulpiride was significantly increased (P<0.05 and P<0.01, respectively; Fig. 6E), as well as in LP tissues treated with 120 mg/kg sulpiride (P<0.05, P<0.01). However, fibronectin protein levels in VP from the 40 mg/kg treated group and VP and DP tissues treated with 120 mg/kg exhibited no significant differences. α-SMA, a specific marker of myofibroblasts, was primarily expressed in the stromal cells of the prostate (Fig. 6C). Compared with controls, α-SMA levels were increased in VP and LP tissues following treatment with 40 mg/kg sulpiride (P<0.05; Fig. 6F); however, levels decreased in LP tissues following the administration of 120 mg/kg sulpiride. Furthermore, α-SMA levels in DP tissues following treatment with 40 mg/kg sulpiride and in VP and DP tissues following 120 mg/kg sulpiride treatment, demonstrated no significant differences (Fig. 6F).

Discussion

The results of the present study revealed the mechanism of sulpiride-induced BPH in male BN rats. Sulpiride stimulates PRL secretion from the pituitary gland and causes prostate toxicity (8). The present study aimed to establish a useful model to assess the mechanism of BPH development and the mechanism of sulpiride-induced BPH in male BN rats.

BPH originates in the transitional and peripheral zones of the humans prostate (46). Murine dorsal and lateral prostate lobes (DLP) are homologous with the peripheral zone in the human prostate and proliferation is more evident in the DLP than in the VP in transgenic mice (47). The current

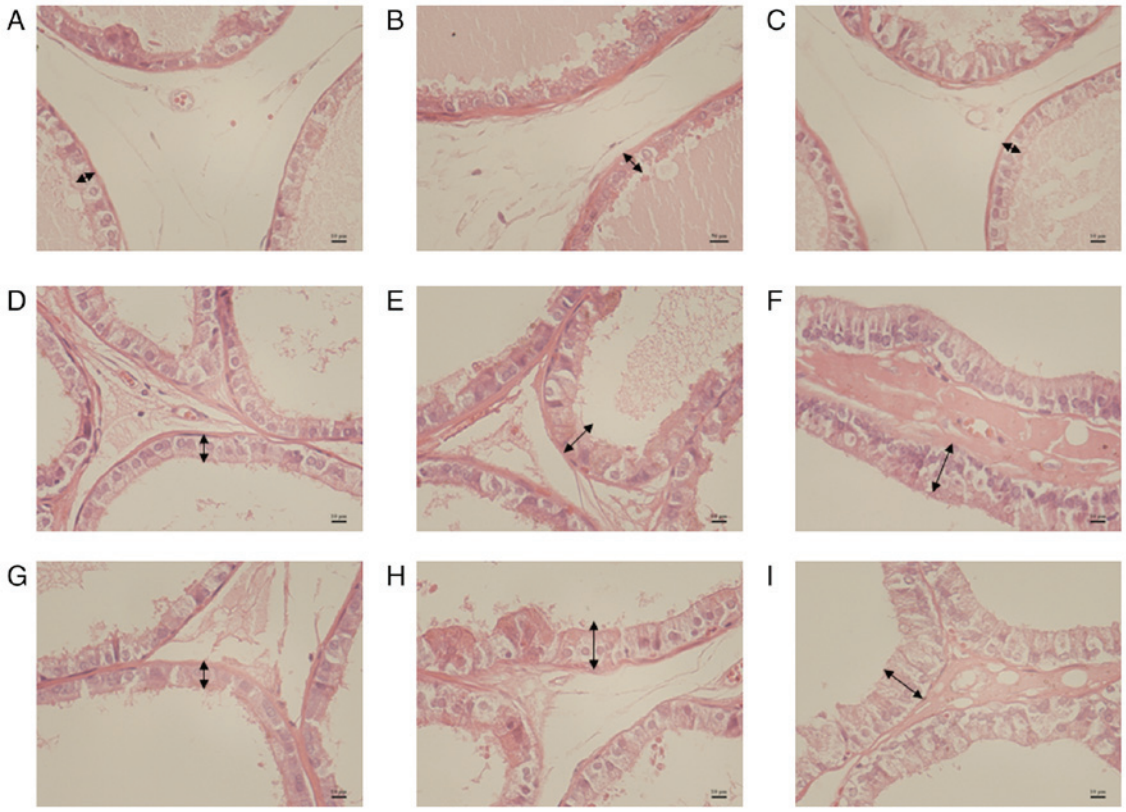

Figure 1. Histological changes in prostate lobes from Brown-Norway rats: Height of prostatic epithelium. Representative sections of comparable regions of the (A) ventral, (B) dorsal and (C) lateral prostatic lobes in the control group. Representative sections of (D) ventral, (E) dorsal and (F) lateral prostatic lobes in rats treated with 40 mg/kg sulpiride. Representative sections of (G) ventral, (H) dorsal and (I) lateral prostatic lobes in the rats treated with 120 mg/kg sulpiride. Magnification, x400. Scale bar, 10 μm. Bidirectional arrows represent the height of the prostatic epithelium.

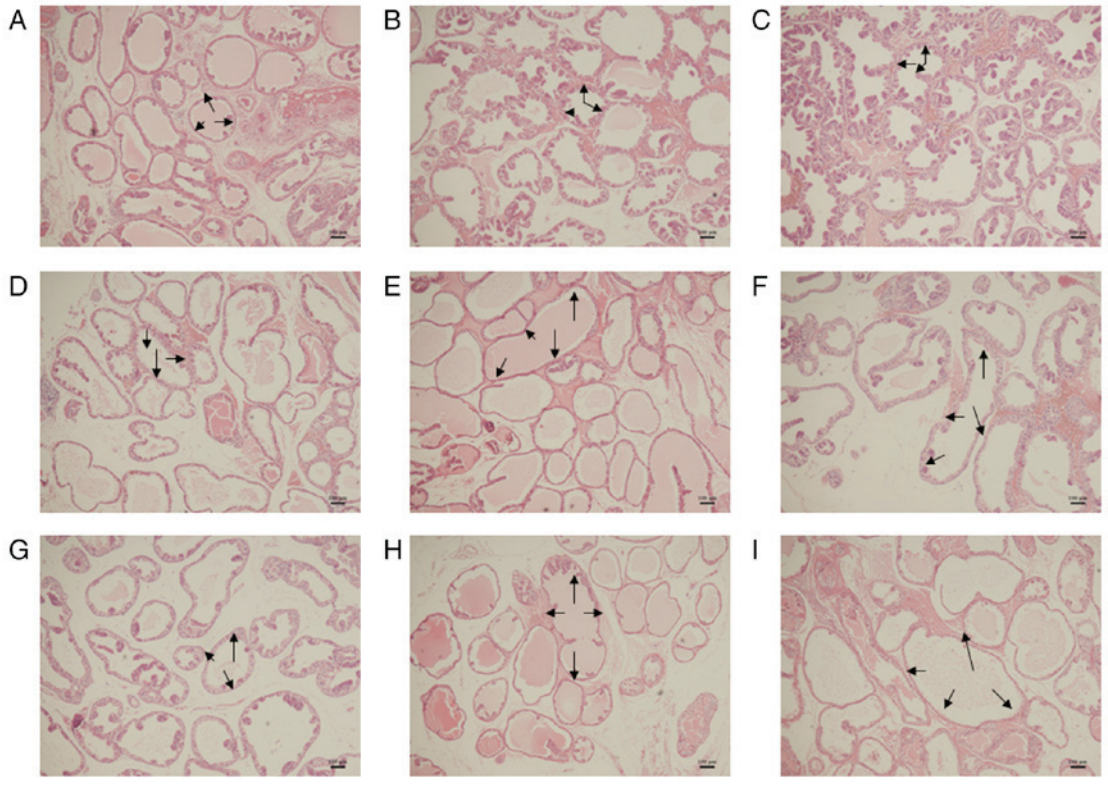

Figure 2. Histological changes in prostate lobes from Brown-Norway rats: Acinar luminal area. Representative sections of comparable regions of the (A) ventral, (B) dorsal and (C) lateral prostatic lobes in the control group. Representative sections of (D) ventral, (E) dorsal and (F) lateral prostatic lobes in rats treated with 40 mg/kg sulpiride. Representative sections of (G) ventral, (H) dorsal and (I) lateral prostatic lobes in the rats treated with 120 mg/kg sulpiride. Magnification, x400. Scale bar, 10 μm. Bidirectional arrows represent the acinar luminal area.

Table IV. Effects of sulpiride on the height of prostatic epithelium of benign hyperplasia prostate modeled Brown-Norway rats.

Experimental group	VP (μm)	DP (μm)	LP (μm)
Control	8.54±1.61	11.41±1.41	13.41±1.71
Sulpiride (40 mg/kg)	14.46±1.49[a]	20.27±3.54[a]	25.14±4.50[a]
Sulpiride (120 mg/kg)	15.92±2.60[a]	19.41±4.05[a]	28.77±3.62[a]

[a]P<0.01 vs. control. VP, ventral prostate; DP, dorsal prostate; LP, lateral prostate. Data were presented as the mean ± standard deviation (n=12).

Table V. Effects of sulpiride on prostate acinar luminal areas of benign hyperplasia prostate modeled Brown-Norway rats.

Experimental group	VP (μm^2)	DP (μm^2)	LP (μm^2)
Control	22,735.78±9,992.60	25,769.59±10,308.27	17,454.82±5,506.35
Sulpiride (40 mg/kg)	36,013.52±8,837.63[a]	66,713.71±6,123.32[a]	88,233.29±7,336.71[a]
Sulpiride (120 mg/kg)	49,829.91±9,301.02[a]	59,617.50±9,814.00[a]	101,443.90±7,374.53[a]

[a]P<0.01 vs. control. VP, ventral prostate; DP, dorsal prostate; LP, lateral prostate. Data were presented as the mean ± standard deviation (n=12).

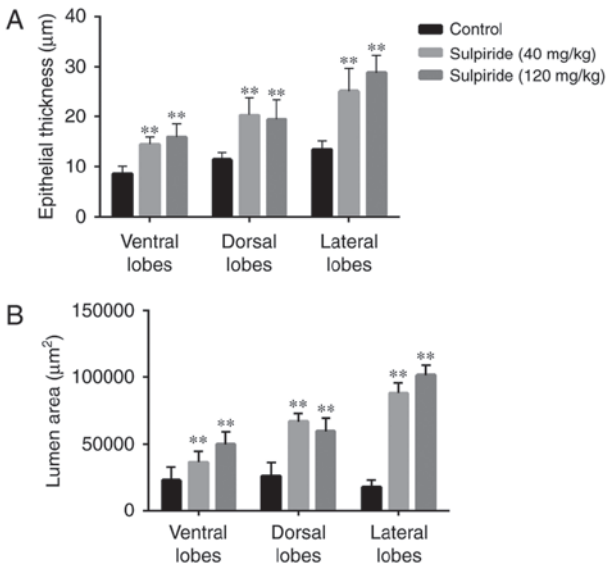

Figure 3. Changes in the prostatic epithelial height and acinar luminal area prostate lobes from Brown-Norway rats. Following treatment with 40 or 120 mg/kg sulpiride for 4 weeks, (A) epithelial height and (B) the acinar lumen area of the lateral prostatic epithelium were observed in rats. **P<0.01 vs. control group.

histological findings suggest that sulpiride significantly induces glandular hyperplasia in LP as follows: Increases of ALA and HPE corroborated experimental findings by Van Coppenolle *et al* (8). Ahonen *et al* (15) demonstrated that PRL stimulates proliferation and acts as an androgen-independent suppressor of apoptosis in prostate epithelial cells, leading to prostate epithelial hyperplasia in DP and LP by using long-term organ cultures of rat prostate tissue. In addition, Słuczanowska-Głąbowska *et al* (9) demonstrated that the columnar and cubical cells of the epithelium were tall and had irregular disposition in sites of hyperplasia of the DP and LP in sexually mature male Wistar rats treated with metoclopramide, while no changes were observed in the VP. As previously mentioned, LP hyperplasia in sulpiride-injected animals may be caused by increased PRL levels (32).

Aside from androgens, the effects of serum PRL on prostate cell differentiation are important (32). It remains unclear whether PRL acts synergistically with or independently of T and current data remains controversial (6,12,48). The results of the present study revealed increased PRL, FSH and T levels in sulpiride-treated groups compared with controls, while LH levels decreased. The present study also demonstrated that PRL and T levels increased, which is in disagreement with the results of the study by Van Coppenolle *et al* (8). This discrepancy may result from the use of different animal species in each study. The results of the present study may also be explained by the reduced activity of 5α-reductase, which can cause increased serum T levels (49). However, this requires further study to confirm. Furthermore, Kindblom *et al* (50) demonstrated that PRL stimulates BPH in mice independently of T. This suggests that PRL affects the prostate and increases T levels, thereby promoting BPH in BN rats, which is similar to the results obtained from Wennbo *et al* (51). Rubin *et al* (52,53) confirmed the prior hypothesis that PRL stimulates T secretion, which reported that PRL accentuates the effects of androgens in the stimulation of prostate growth and function. Additionally, Van Coppenolle *et al* (8) demonstrated that T levels are higher in controls compared with animals treated with sulpiride, which is not congruent with the aforementioned results. Previous studies have indicated that an increase in PRL inhibits gonadotrop-releasing hormone (GnRH) release from the hypothalamus and reduces LH release via a negative feedback regulatory mechanism (54,55). The structure and function of the prostate are influenced by the hypothalamus-pituitary-gonadal axis (56), while tissue growth and hormone secretion are primarily affected by T regulation (57). In turn, T secretion is controlled by LH and

Table VI. Plasma PRL, FSH, T and LH levels.

Experimental group	PRL (ng/ml)	FSH (ng/ml)	T (pg/ml)	LH (mIU/ml)
Control	5.87±3.16	4.82±1.67	197.27±170.63	701.58±515.83
Sulpiride (40 mg/kg)	57.48±15.52[b]	7.46±2.98[b]	535.07±352.90[b]	575.03±123.69[b]
Sulpiride (120 mg/kg)	106.82±27.2[b]	5.36±1.00	273.16±92.44[a]	367.61±265.64[b]

PRL, prolactin; FSH, follicle-stimulating hormone; T, testosterone; LH, luteinizing hormone. Data were presented as the mean ± standard deviation (n=12). [a]P<0.01 and [b]P<0.001 vs. controls.

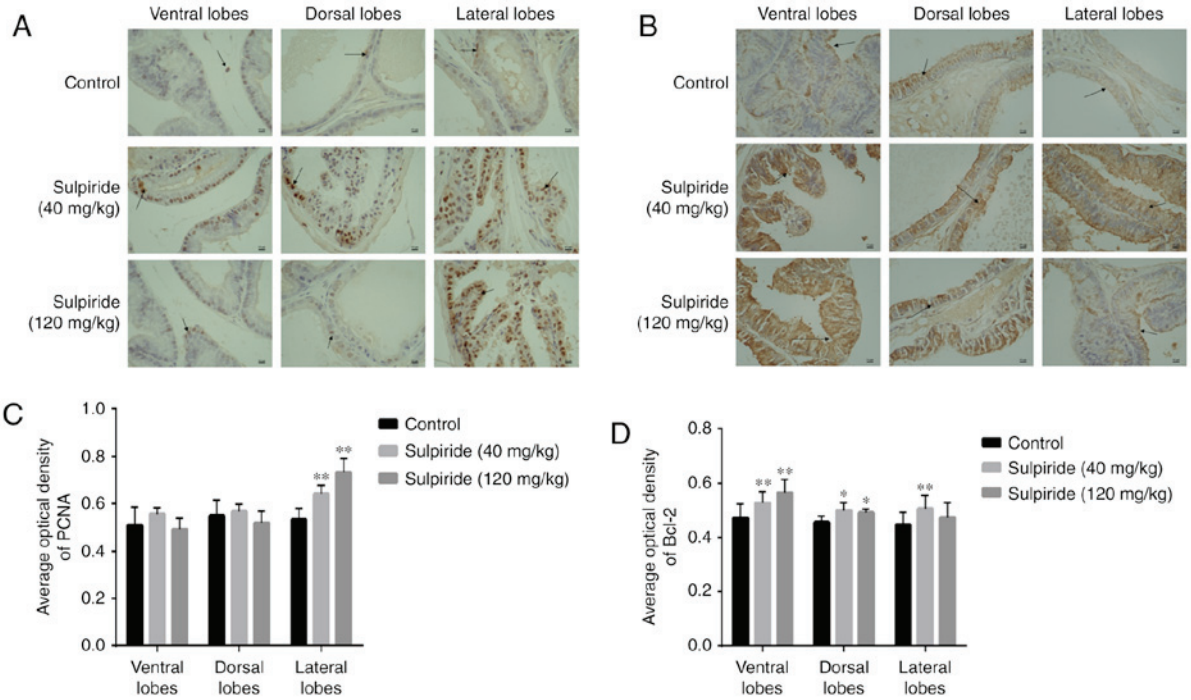

Figure 4. Immunohistochemical localization of (A) PCNA and (B) Bcl-2 in tissue sections of prostatic lobes in sulpiride treated Brown-Norway rats. Black arrows indicate strong positive PCNA and Bcl-2 signals. Semi-quantitative analysis of (C) PCNA and (D) Bcl-2 staining. Magnification, x400. Scale bar, 10 μm. *P<0.05 and **P<0.01 vs. control group. PCNA, proliferating cell nuclear antigen; Bcl-2, B-cell lymphoma-2.

FSH (58,59). Sulpiride has an effect on the hypothalamic tuberoinfundibular dopaminergic neurons at the pituitary and increases PRL release (60). Furthermore, PRL stimulates the release of endogenous opioids, which strongly inhibits GnRH release (61). LH was downregulated and FSH was upregulated via the feedback regulation mechanism utilized by the hypothalamus-pituitary-gonadal axis; elevated FSH stimulates the release of T, which in turn inhibits GnRH release.

The results of the present study indicate that sulpiride promotes prostate growth in BN rats by increasing PRL and T levels. In addition, synergistic effects between PRL and T were demonstrated. PCNA is used as a proliferation marker in the rat prostate (28). IHC results in the present study revealed that PCNA was primarily expressed in the nucleus of epithelial cells. In addition, PCNA expression in LP samples was significantly increased following sulpiride treatment compared with the control group. However, PCNA levels were unchanged in VP and DP samples. A previous study has indicated that the VP and DP in BN rats are insensitive to PRL, no PCNA overexpression was detected in the present study (47). It has been demonstrated that Bcl-2 reduces cell apoptosis in prostate tissues (62). As revealed by IHC in the present study, the Bcl-2 protein was primarily expressed in the cytoplasm of epithelial cells and its levels increased significantly in rats treated with sulpiride with the exception of LP at sulpiride 120 mg/kg. Van Coppenolle *et al* (8) demonstrated that Bcl-2 expression is increased in the LP of sulpiride treated rats. In the present study, LP hyperplasia may be explained by the PRL-induced proliferation as mediated by PCNA overexpression and apoptosis inhibition via Bcl-2 overexpression. These experimental results suggest that sulpiride treatment promotes proliferation and inhibits epithelial cell apoptosis, promoting prostate epithelial hyperplasia.

Estrogen receptors belong to the nuclear receptor family of proteins that includes ERα and ERβ; the former serves a role in the promotion of prostate cell proliferation and the latter exerts beneficial effects by repressing prostate growth (63). Sulpiride competes with estradiol for the ERα binding due to its structural similarity and binds specific DNA sequences to induce target genes following nuclear entry (64). As demonstrated in

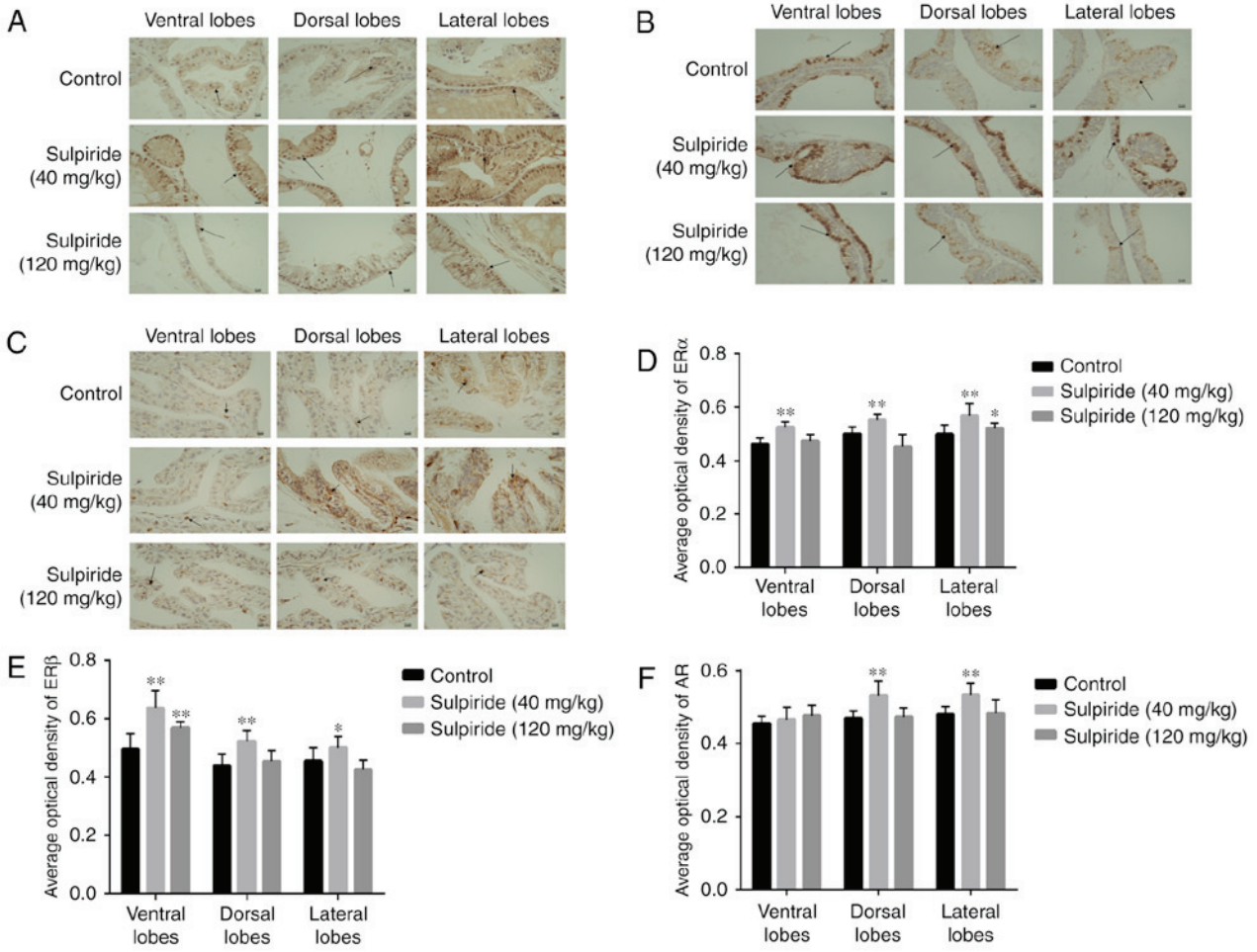

Figure 5. Immunohistochemical localization of (A) ERα, (B) ERβ and (C) AR in sulpiride treated Brown-Norway rat prostate tissues. Black arrows indicate strong positive ERα, ERβ and AR signals. Semi-quantitative analysis of (D) ERα, (E) ERβ and (F) AR staining. Magnification, x400. Scale bar, 100 μm. *P<0.05 and **P<0.01 vs. control group. ERα, Estrogen receptor-α; ERβ, Estrogen receptor-β; AR, androgen receptor.

the present study, ERα and ERβ are primarily expressed in the nucleus of epithelial cells. In sulpiride treatment groups, ERα levels in LP tissues were significantly increased compared with control values. The 40 mg/kg sulpiride group significantly increased ERα levels in different lobes. The prostate is an androgen-dependent organ and AR serves a pivotal role in the regulation of its function, growth and differentiation (65). The transcription factor AR is activated by its ligand, binding a specific androgen response element to promote transcription (33). In the present study, AR was expressed in the nucleus and cytoplasm of epithelial cells, with levels increasing in VP with the dosage of 120 mg/kg sulpiride. However, 40 mg/kg sulpiride group significantly increased AR in DP and LP tissues, more so than the 120 mg/kg dosage. The expression trend of AR was similar to that of ERα, but opposite to that of ERβ. Higher AR expression in the lateral lobes of BN rats may be explained by the following mechanisms. Banerjee *et al* (66) reported that AR expression is not dependent on T, as AR levels remain unchanged in castrated rats. In addition, Sluczanowska-Glabowska *et al* (9) demonstrated that AR is elevated in LP tissues of the experimental group, but lower in the VP and DP tissues. Prins *et al* (67) revealed that increased PRL leads to AR upregulation in the LP of experimental animals. Furthermore, Holland and Lee (68) demonstrated that increased serum PRL and decreased T results in the increased sensitivity of the LP to PRL. As demonstrated in the present study, ERα and AR levels were increased following sulpiride treatment, indicating that sulpiride causes LP hyperplasia of the prostate via the ERα and AR signaling pathways.

The present study evaluated the expression of stromal cell biomarkers following sulpiride administration. The stromal compartment contains fibroblasts, vasculature, nerves and immune components (69,70). Mesenchymal cells consist of fibroblasts, myofibroblasts and smooth muscle cells (71). Mesenchymal cell markers, including vimentin, α-SMA and fibronectin, are associated with the development of prostate tissues (72-76). As demonstrated by IHC in the present study, vimentin, fibronectin and α-SMA were primarily expressed in mesenchymal cells. In addition, vimentin expression in LP was significantly increased following the administration of 40 and 120 mg/kg sulpiride. These findings suggest that sulpiride also causes hyperplasia in the stromal component of LP, indicating that BPH is a proliferative stromal disease, similar to spontaneous prostatic hyperplasia (4).

PCNA as a proliferation marker and Bcl-2 as an anti-apoptotic marker mediate cell proliferation and apoptosis, respectively. The present study demonstrated that the expression of PCNA and Bcl-2 was upregulated in LP of 40 mg/kg

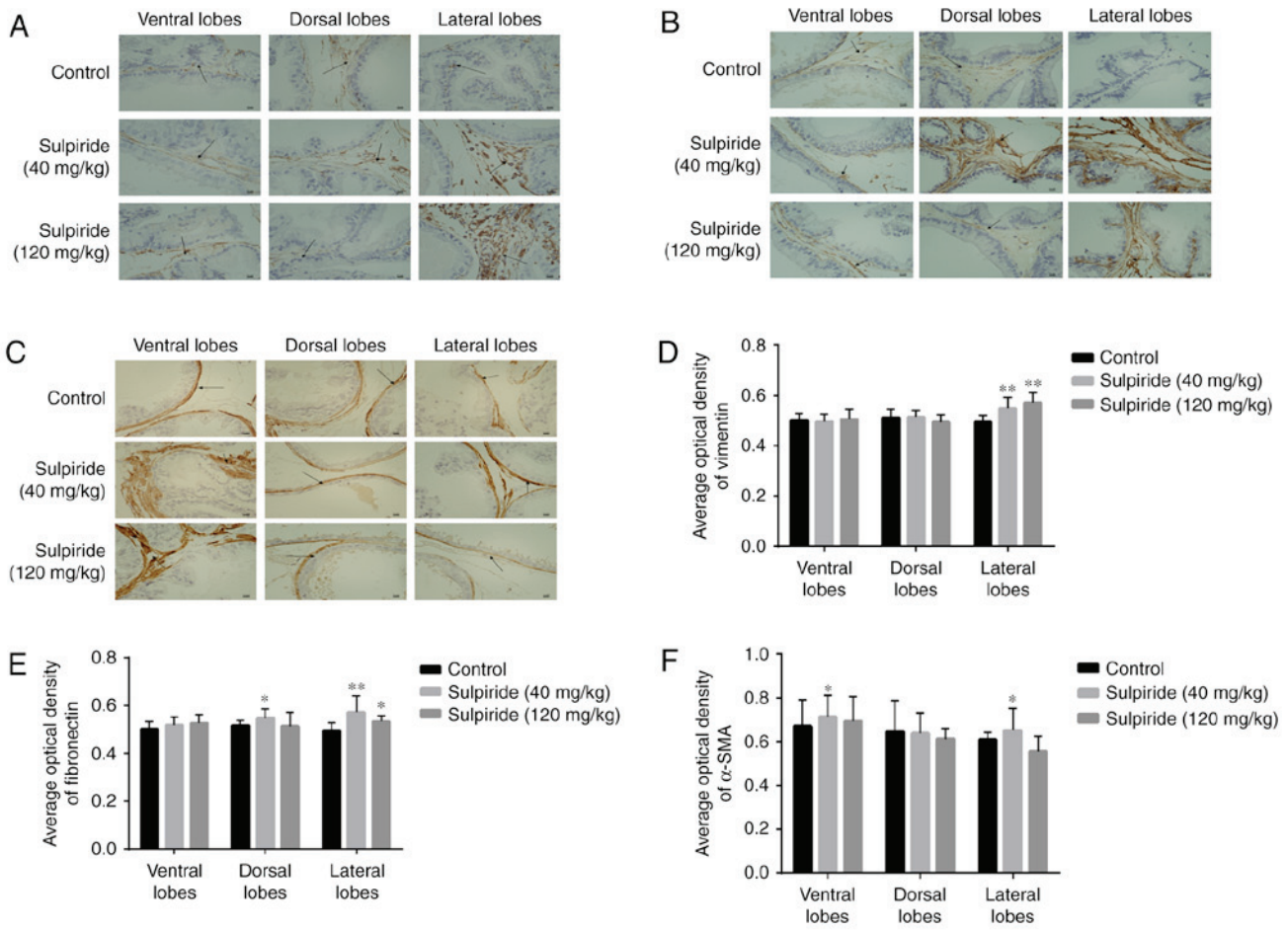

Figure 6. Immunohistochemical localization of (A) vimentin, (B) fibronectin and (C) α-SMA in sulpiride treated Brown-Norway rat prostate tissues. Black arrows indicate strong positive vimentin, fibronectin and α-SMA signals. Semi-quantitative analysis of (D) vimentin, (E) fibronectin and (F) α-SMA staining. Magnification, x400. Scale bar, 100 μm. *P<0.05 and **P< 0.01 vs. control group. α-SMA, α-smooth muscle actin.

sulpiride group. Van Coppenolle *et al* (8) demonstrated that PRL stimulates Bcl-2 expression in the prostate gland of sulpiride-treated Wistar rats and inhibits prostate cell apoptosis. The results of the present study revealed that BN rats treated with sulpiride exhibited higher PRL levels compared with control rats. It was hypothesized that higher Bcl-2 levels caused by higher PRL inhibits prostate cell apoptosis except for the LP tissues at sulpiride 120 mg/kg.

A previous study has determined that vimentin, an important intermediate fibrin protein in cells, functions as a general intermediate fiber and participates in the process of cell apoptosis (77). Morishima *et al* (78) revealed that apoptosis was accompanied by vimentin fragmentation. Fibronectin serves an important role in cell migration, adherence and proliferation. Wu *et al* (79) demonstrated that a lack of fibronectin triggers mesangial apoptosis via the intrinsic pathway. In the present study it was determined that sulpiride promotes the expression of stromal cell biomarkers vimentin and fibronectin in BN rats. Based on these results, it was hypothesized that sulpiride may inhibit the apoptosis of prostate cells by upregulating vimentin and fibronectin.

The aforementioned results of the current study indicate that sulpiride promotes proliferation and inhibits prostate cell apoptosis by upregulating PCNA, Bcl-2 and stromal cell biomarker (vimentin and fibronectin) expression.

The present study established a prostate hyperplasia model in BN rats treated with sulpiride (40 mg/kg/day), the results of which indicate that sulpiride causes LP hyperplasia in BN rats by promoting proliferation and inhibiting apoptosis in prostate cells, likely via ERα and AR signaling. However, further study is required to fully elucidate the possible mechanisms by which sulpiride activates ERα and AR functions and the pathways involved.

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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZS and YL designed the study. CZ performed the experiments and analyzed the experimental data. DC, CS, DH and JZ participated in the animal experiments. YC performed the ELISA experiments. XM and LL performed the tissue specimen preparation. All authors have read and approved this manuscript.

Ethics approval and consent to participate

The present study was approved by the Shanghai Institute of Planned Parenthood Research Animal Care (Shanghai, China). All animal procedures were approved by the Animal Care and Use Committee of Shanghai Institute of Planned Parenthood Research (Shanghai, China) and performed according to the Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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