

Additive effect of oral LDD175 to tamsulosin and finasteride in a benign prostate hyperplasia rat model

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Objective: We investigated the benefits of the BK_{Ca} agonist 4-chloro-7-trifluoromethyl-10H-benzo[4,5]furo[3,2-b]indole-1-carboxylic acid (LDD175) combined with tamsulosin and finasteride, in a benign prostatic hyperplasia (BPH) rat model.

Materials and methods: Castration was performed by bilateral orchiectomy under ketamine anesthesia. A rat model of BPH was established by daily intramuscular administration of testosterone propionate plus 17 β -estradiol for 8 weeks. Model rats were administered combinations of 20 mg/kg LDD175, 0.01 mg/kg tamsulosin and 1 mg/kg finasteride once daily by oral gavage for 4 weeks from week 6 to 9 post-surgery. Intraurethral pressure induced by electrostimulation of the hypogastric nerve was measured at the end of administration. Body and genitourinary organ weights were recorded, serums were assayed for hormone concentrations, and tissues were subjected to histopathology, and analyses of α 1-adrenoceptor mRNA and protein expression levels after treatment.

Results: Combined LDD175, tamsulosin, and finasteride significantly decreased prostatic index, serum hormone levels, epithelial thickness, and prostate expression of α 1-adrenoceptors in BPH model rats. The 3-drug combination was more effective than any other combination or LDD175 alone.

Conclusion: These results suggest that LDD175 addition to tamsulosin and finasteride may be beneficial for the treatment of BPH patients who do not respond to tamsulosin plus finasteride.

Keywords: α 1-adrenoceptors, α 1-adrenergic receptor antagonists, benzofuroindole, intraurethral pressure, 5 α -reductase inhibitors

Introduction

Benign prostatic hyperplasia (BPH), also known as benign enlargement of the prostate, is a hormone and age-related disease characterized by histological changes in the prostate gland and variable enlargement of the prostate.¹ Prostate enlargement induces various symptoms, including urinary urgency, slow stream, nocturia and increased daytime frequency.² These symptoms have a considerable negative effect on the quality of life of BPH patients.^{3,4} Although the pathogenesis of BPH has not been fully elucidated, it involves hormonal changes in an aging man.⁵ The development and growth of normal prostate mainly depends on androgen stimulation, by dihydrotestosterone (DHT) that is a highly active metabolite of testosterone synthesized from the prostate 5 α -reductase enzyme.^{6,7}

For patients with BPH, 2 main treatment options exist: α 1-adrenergic receptor antagonists to reduce smooth muscle tone in the prostate and the bladder neck,

and 5 α -reductase inhibitors to reduce prostate size.⁸ Tamsulosin and finasteride have been the most popular medication prescribed for treating BPH.⁹ McConnell et al¹⁰ reported that only 64% of men receiving both therapies showed the reduced risk of clinical progression, defined as worsening of symptoms, acute urinary retention, incontinence and urinary tract infection. Furthermore, these drugs induce undesirable side effects, including decreased libido, erectile dysfunction, dizziness, postural hypotension, asthenia, and occasional syncope.^{11,12} Therefore, it is highly desirable to develop an α 1-adrenergic antagonist or other medication that can selectively suppress the smooth muscle tone of lower urinary tract without vascular effects and decrease prostate volume without sexual dysfunction for the treatment of urinary outlet obstruction in BPH.¹³

Activation of large-conductance Ca²⁺-activated K (BK_{Ca}) channels decreases vascular smooth muscle tone under physiological conditions.¹⁴ However, the major limitations of classical BK_{Ca} channel opener compounds are weak potency and insufficient selectivity.¹⁵ Recently, Gormemis et al¹⁶ found the new benzofuroindole derivative, LDD175, which showed remarkable potency to activate macroscopic Slo BK_{Ca} channels. The toxic effect of LDD175 is not well known. The oral administration of LDD175 (10 and 100 mg/kg) produced no clinical signs or adverse effects.¹⁷

The purpose of this investigation was to evaluate that addition of oral LDD175 to conventional tamsulosin plus finasteride treatment can augment pharmacological efficacy in a BPH rat model.

Materials and methods

Chemicals and reagents

Testosterone was purchased from Wako-Reagent (Tokyo, Japan). Finasteride and 17 β -estradiol were purchased from Sigma-Aldrich (St Louis, MO, USA). Tamsulosin was donated by ILDONG Pharmaceutical Company (Seoul, Republic of Korea) and LDD175 was kindly provided by AnyGen Company (Gwangju, Republic of Korea). All other chemicals were purchased from standard suppliers. Testosterone plus 17 β -estradiol used in this study was dissolved in corn oil. LDD175 was dissolved in 10% Tween 20 buffer.

Treatment of BPH rat model with LDD175, tamsulosin and finasteride

All animal procedures in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Chonbuk National University and were approved by the Institutional Animal Care and Use Committee of

Chonbuk National University Laboratory Animal Center (CBNU 2015-0012). A total of 42 sexually male SD rats (250–300 g) were selected for this study.

The protocol to induce BPH was slightly modified from that of Suzuki et al.¹⁸ The 6 rats were incised above the pelvic region on the ventral side and then sutured without cutting off the testicles as a control group (CON+Vehicle). The testicles of 36 male SD rats were removed under anesthesia with intraperitoneal ketamine (50 mg/kg; Bayer, Ansan, Republic of Korea) and 2% xylazine hydrochloride (25 mg/kg; Bayer). The 6 castrated rats were intramuscularly administered corn oil (CAS+Vehicle). A week after castration, 30 rats were intramuscularly administered testosterone (3 mg/kg) plus 17 β -estradiol (0.03 mg/kg) daily for 8 weeks to induce BPH. The 30 castrated BPH rats were then randomly assigned to 5 experimental groups: disease control group (BPH+Vehicle), LDD175-treated (BPH+L), LDD175 and tamsulosin-treated (BPH+LT), LDD175 and finasteride-treated (BPH+LF) and LDD175, tamsulosin and finasteride-treated (BPH+LTF). Treatment groups received the indicated combination of LDD175 (20 mg/kg), tamsulosin (0.01 mg/kg) and/or finasteride (1 mg/kg) once daily for 4 weeks from week 6 to 9 post-surgery. The volumes of administration were 6 mL/kg for oral administration and 0.7 mL/kg for intramuscular injection, respectively. The volumes were calculated based on recent weights.

Recording of intraurethral pressure (IUP) and blood pressure (BP)

After the treatment period, the IUP in rats was measured according to the method described previously.¹⁹ Briefly, the bladder and prostate were exposed through a midline incision in the abdomen. The bladder was cut through the dome and a polyethylene tube (SP45, Natsume, Tokyo, Japan) was inserted through the opening toward the bladder neck. Normal saline was continuously infused into the intraurethral lumen through the tube at a constant rate of 0.5 mL/10 minutes using a syringe pump (KD Scientific Inc, Holliston, MA, USA). The infusion pressure signals from the urethra were measured using a pressure transducer (Living Systems Instrumentation, St Albans City, VT, USA), amplified and recorded using a PowerLab data 400 acquisition computer system (Software Chart, AD Instrument, Sydney, NSW, Australia).

The hypogastric nerves were field-stimulated electrically (pulse duration 1 ms, voltage 5 V, frequency 5, 10, or 20 Hz) using a Grass stimulator (S48, Grass Telefactor, Warwick, RI, USA). Electrostimulation (EST) at each frequency was continued for 30 seconds. The left common carotid artery

was cannulated with a polyethylene tube (SP45, Natsume, Tokyo, Japan) in order to monitor systemic arterial pressure with a pressure transducer (Living Systems Instrumentation) placed at the heart level.

Sample collection

Blood was obtained from the abdominal vein. Organs such as the prostate, bladder, penis and seminal vesicles were surgically removed. Prostate volume was measured and the prostatic index was calculated as prostate volume/body weight $\times 100$. One piece of prostate tissue was collected from the same position in every rat and fixed with 3.7% formalin for histopathological analyses.

Measurement of hormone levels in the serum

Serum levels of DHT, testosterone, free testosterone, and estradiol were measured using commercial kits (Rat DHT ELISA Kit, CUSABIO, Wuhan, China; TESTO-CT2 Kit, Cisbio Inc., Bedford, MA, USA). All protocols were performed according to the manufacturer's instructions.

Histopathological examination

Fixed prostate tissues embedded in paraffin wax were cut into 4 μm thick sections and stained with hematoxylin (Sigma-Aldrich, MHS-16) and eosin (Sigma-Aldrich, HT110-1-32). The sections were mounted and cover-slipped using mounting medium (Invitrogen, Carlsbad, CA, USA) and then examined under a microscope (Zeiss, Oberkochen, Germany).

Quantitative real-time polymerase chain reaction (RT-PCR) analysis for $\alpha 1$ -adrenoceptors

Total RNA was extracted from prostate using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA) and reverse transcribed into cDNA using a high-capacity cDNA reverse transcription kit (AccuPower[®] Rocketscript[™] Cycle RT Pre-mix, BIONEER, Daejeon, Republic of Korea). Gene-specific primers used for PCR amplification were as follows: rat $\alpha 1A$ -adrenoceptors 5'-GGGCTACTGGCCTTTGG-3' (forward) and 5'-CTGTGCAGCAT AAGACGTCCAC-3' (reverse), rat $\alpha 1D$ -adrenoceptors 5'-TCTTTCCTTGACTCCGCA-3' (forward) and 5'-GCCGTGAAGGTCCCAATG-3' (reverse) and β -actin 5'-TG GGCCGCCCTAGGCAC-CA-3' (forward) and 5'-CGGTTGGCCTTAGGGTTCAG-3' (reverse). Expression levels of target were calculated relative to β -actin expression. The real-time PCR reaction contained in a final volume of 20 μL , 1 μg of reverse transcribed total

RNA and 2 \times PCR master mix. PCR reaction was carried out in 96-well plates using the Exicycler TM 96 Real-Time Quantitative Thermal Block (BIONEER). All the reactions were under the same cycling conditions: 10 minutes at 95°C; 40 cycles of 5 seconds at 95°C, 25 seconds at 58°C, 30 seconds at 72°C, and 5 seconds at 65°C. All reactions were done in triplicate.

Western blotting for expression of $\alpha 1$ -adrenoceptors

Prostate tissues were minced into small pieces and homogenized in lysis buffer containing 0.32 M sucrose, 0.2 M HEPES (pH 7.4), 1 nM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 10 $\mu\text{g}/\text{mL}$ leupeptin, 2 $\mu\text{g}/\text{mL}$ aprotinin, 1 $\mu\text{g}/\text{mL}$ pepstatin, 10 $\mu\text{g}/\text{mL}$ trypsin inhibitor and 1 mM phenylmethylsulfonyl fluoride. The homogenized solution was placed on ice for 15 minutes and centrifuged at 13,000 rpm for another 15 minutes at 4°C. The supernatant was separated. Bovine serum albumin was used for the separation of solution. Lysate protein (20 μg) was denatured at 95°C for 5 minutes and electroblotted onto 0.2 μm PVDF membranes (Amersham Biosciences, Piscataway, NJ, USA). Membranes were reacted with $\alpha 1A$ and 1D-adrenoceptor antibodies (GeneTex, San Antonio, TX, USA) for 2 hours. As secondary antibodies, anti-mouse IgG-HRP for $\alpha 1A$ and 1D-adrenoceptor (1:5,000 dilution; Zymed Laboratories, San Francisco, CA, USA) were reacted at ambient temperature for 1 hour, and the membrane was washed again with Tween tris buffered saline (TTBS) 6 times, with 5 minutes intervals between each washing. Chemiluminescence was detected using enhanced chemiluminescence (ECL) Western blotting detection reagents. Beta-actin was used as the control.

Statistical evaluation

All analyses were performed using SPSS version 12.0 (SPSS Institute, Chicago, IL, USA). Values are expressed as mean \pm SD. Differences among treatment group means were tested by analysis of variance and post-hoc Duncan's multiple range tests. A *P*-value < 0.05 was considered statistically significant for all tests.

Results

Effects of LDD175, tamsulosin and finasteride combinations on body and genitourinary organ weights

Body weight at 1 week post-castration did not differ among the groups (Table 1). However, body weight at 9 weeks post-castration was significantly lower in the disease control group compared to the castration group (CAS+Vehicle)

Table 1 Changes in weights of body and genitourinary organs

Group	Body weights (g)		Bladder (g)	Penis (g)	Seminal vesicle (g)	Prostate volumes	
	1 week	9 week				Absolute volume (g)	Prostatic index
CON+Vehicle	329.33±24.73	423.10±13.40 ^a	0.13±0.04 ^c	0.43±0.08 ^{cd}	0.41±0.07 ^d	0.88±0.25 ^d	0.21±0.06 ^c
CAS+Vehicle	331.57±13.99	415.05±06.74 ^a	0.09±0.02 ^{bc}	0.24±0.07 ^e	0.13±0.06 ^e	0.15±0.04 ^e	0.04±0.01 ^d
BPH+Vehicle	336.00±08.53	349.45±12.57 ^b	0.23±0.06 ^a	0.44±0.05 ^a	0.75±0.14 ^a	1.98±0.30 ^a	0.57±0.14 ^a
BPH+L	347.20±04.55	354.01±09.74 ^b	0.20±0.07 ^{ab}	0.53±0.07 ^{ab}	0.66±0.08 ^b	1.42±0.08 ^b	0.40±0.03 ^b
BPH+LT	339.00±12.49	348.55±08.53 ^b	0.18±0.09 ^{ab}	0.52±0.08 ^{bc}	0.65±0.10 ^{bc}	1.44±0.13 ^b	0.41±0.05 ^b
BPH+LF	339.80±22.53	352.31±10.12 ^b	0.14±0.04 ^{bc}	0.41±0.05 ^{cd}	0.59±0.13 ^{bc}	1.39±0.11 ^b	0.39±0.02 ^b
BPH+LTF	330.83±23.58	355.78±23.74 ^b	0.14±0.03 ^{bc}	0.46±0.12 ^d	0.56±0.10 ^c	1.12±0.12 ^c	0.34±0.05 ^b

Notes: Values with different superscript alphabets in the same row are significantly different ($P<0.05$) by one-way analysis of variance and the Duncan's multiple range tests. **Abbreviations:** BPH, benign prostatic hyperplasia; BPH+Vehicle, disease control; BPH+L, LDD175 (20 mg/kg); BPH+LT, LDD175 and tamsulosin (0.01 mg/kg); BPH+LF, LDD175 and finasteride (1 mg/kg); BPH+LTF, LDD175, tamsulosin, and finasteride; CAS+Vehicle, castration; CON+Vehicle, control.

and the sham-operated control group (CON+Vehicle). The absolute prostate volume and prostatic index were significantly lower in the BPH+L group than the disease control group and lower still in the group receiving all three drugs (BPH+LTF group).

Effects of LDD175, tamsulosin and finasteride combinations on serum hormones

Serum DHT, testosterone, free testosterone, and estradiol levels are shown in Figure 1. Serum DHT was markedly

higher in the disease control group (4.70 ± 0.19 ng/mL) than the CON+Vehicle group (Figure 1A). However, DHT levels were significantly lower in the BPH+L group (4.06 ± 0.59 ng/mL) and lower still in the BPH+LTF group (2.97 ± 0.55 ng/mL) compared with the disease control group. The disease control group also exhibited significantly increased serum testosterone (15.66 ± 2.79 ng/mL) compared with the CON+Vehicle group (3.31 ± 1.05 ng/mL; Figure 1B). In contrast, serum testosterone levels were significantly lower in the BPH+L and BPH+LTF groups compared with the disease control group. The level of free testosterone was

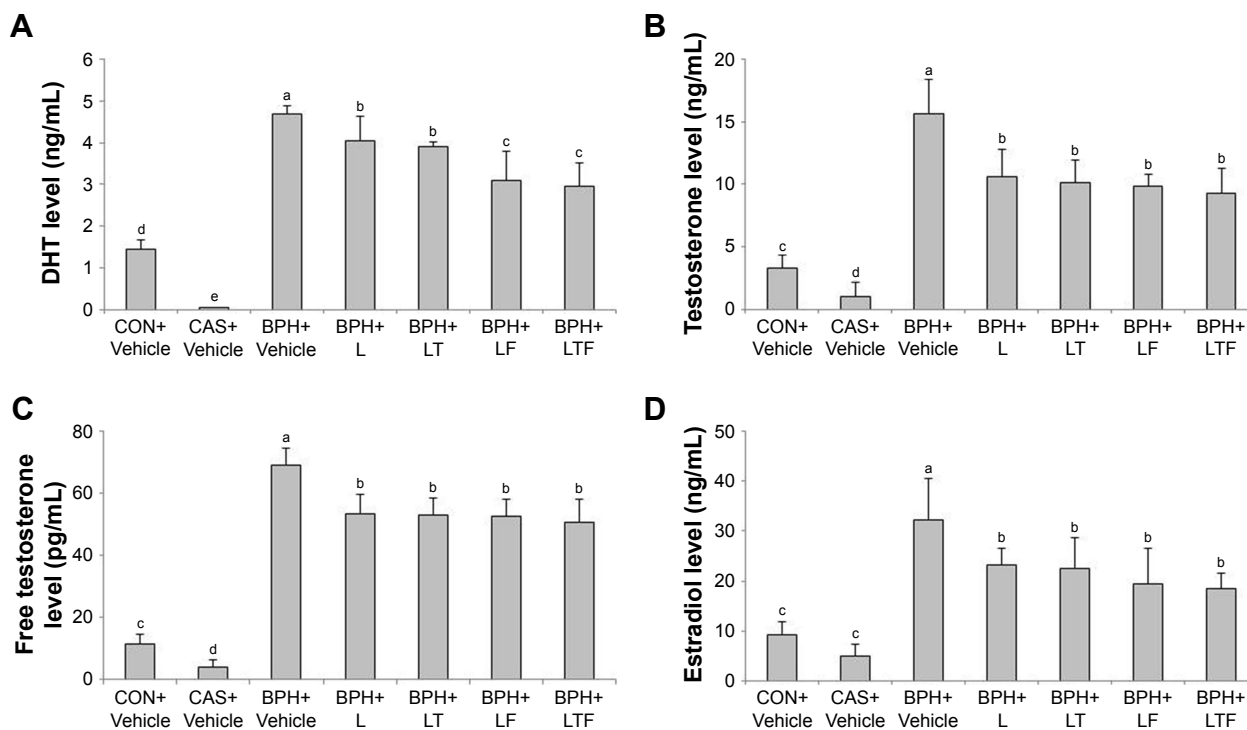


Figure 1 Effects of LDD175, tamsulosin, and finasteride combinations on serum hormone levels in each treatment group.

Notes: (A) DHT. (B) Testosterone. (C) Free testosterone. (D) Estradiol. Values with different superscript alphabets in the same row are significantly different ($P<0.05$) by one-way analysis of variance and the Duncan's multiple range tests.

Abbreviations: BPH, benign prostatic hyperplasia; DHT, dihydrotestosterone; BPH+Vehicle, disease control; BPH+L, LDD175 (20 mg/kg); BPH+LT, LDD175 and tamsulosin (0.01 mg/kg); BPH+LF, LDD175 and finasteride (1 mg/kg); BPH+LTF, LDD175, tamsulosin, and finasteride; CAS+Vehicle, castration; CON+Vehicle, control.

significantly higher in the disease control group compared with the CON+Vehicle group (69.29 ± 5.52 pg/mL versus 11.58 ± 3.15 pg/mL; Figure 1C), but was reduced in the BPH+L group (53.38 ± 6.46 pg/mL) and slightly further in the BPH+LTF group (50.62 ± 7.64 pg/mL). The disease control group also exhibited significantly increased serum estradiol compared with the CON+Vehicle group (32.19 ± 8.47 ng/mL versus 9.31 ± 2.51 ng/mL; Figure 1D). Serum estradiol was significantly lower in both BPH+L and BPH+LTF groups compared with the disease control group, with a greater reduction in the BPH+LTF group than the BPH+L group.

Effects of LDD175, tamsulosin, and finasteride combinations on prostatic epithelial hyperplasia

Prostatic epithelial thickness was significantly greater in the disease control group compared with the CON+Vehicle

group, while the BPH+L group showed only mild epithelial hyperplasia compared with the disease control group (Figure 2A). The BPH+LTF group also exhibited markedly reduced hyperplasia compared with the disease control group (Figure 2B). Epithelial thickness of the prostate was significantly reduced in the BPH+LTF group compared with the disease control group (32.61 ± 5.48 μ m versus 63.16 ± 6.28 μ m) and the LDD175, tamsulosin, and finasteride combination was the most effective treatment for reducing prostatic epithelial hyperplasia.

Effects of LDD175, tamsulosin and finasteride combinations on IUP and BP

The IUP responses to EST were frequency-dependent (Figure 2C). At all 3 frequencies applied, the responses to EST were significantly higher in the disease control group than the CON+Vehicle group. The BPH+L group

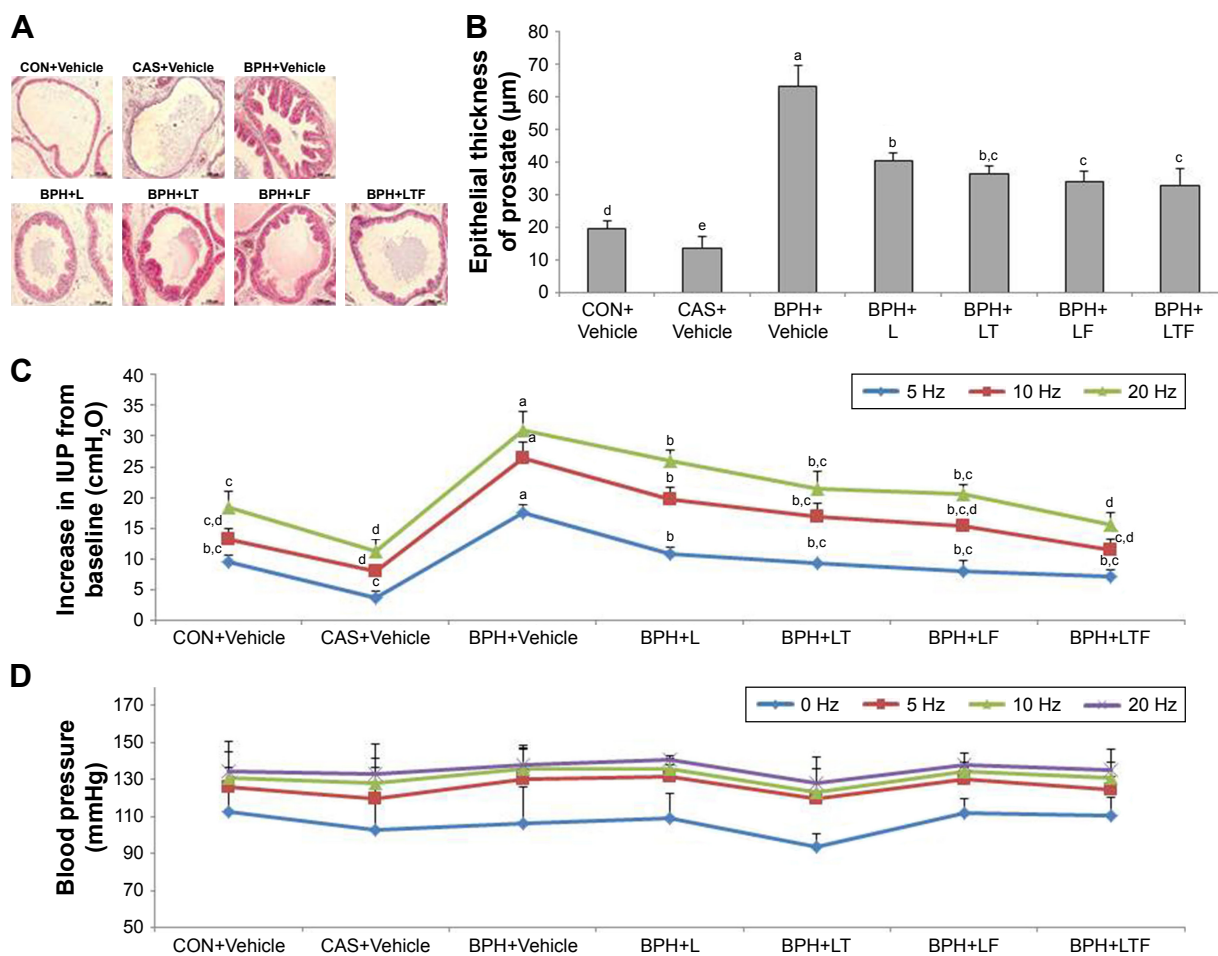


Figure 2 Effects of LDD175, tamsulosin, and finasteride combinations on prostate histopathology and IUP response to EST of the hypogastric nerve.

Notes: (A) Histological changes in the prostate. Original magnifications 20 \times . (B) Epithelial thickness of the prostate. (C) Effects on the IUP response to EST. (D) Effect on blood pressure (BP). Values with different superscript alphabets in the same row are significantly different ($P < 0.05$) by one-way analysis of variance and the Duncan's multiple range tests.

Abbreviations: BPH, benign prostatic hyperplasia; BPH+Vehicle, disease control; BPH+L, LDD175 (20 mg/kg); BPH+LT, LDD175 and tamsulosin (0.01 mg/kg); BPH+LF, LDD175 and finasteride (1 mg/kg); BPH+LTF, LDD175, tamsulosin, and finasteride; CAS+Vehicle, castration; CON+Vehicle, control; EST, electrostimulation; IUP, intraurethral pressure.

demonstrated reduced EST frequency-dependent IUP elevation. The increase in IUP from baseline was significantly lower in the BPH+LTF group compared with the disease control group at all frequencies tested (7.11 ± 1.09 cmH₂O versus 17.48 ± 1.32 cmH₂O at 5 Hz; 11.51 ± 1.70 cmH₂O versus 26.51 ± 2.45 cmH₂O at 10 Hz; 15.72 ± 1.93 cmH₂O versus 31.02 ± 2.91 cmH₂O at 20 Hz). The IUP elevation was smaller in the BPH+LTF group than the BPH+L group at all frequencies. In contrast, there were no significant differences in BP among the groups (Figure 2D).

Effects of LDD175, tamsulosin, and finasteride combination on $\alpha 1$ -adrenoceptor subtype mRNA expression in prostate tissue

The expression levels of $\alpha 1$ -adrenoceptor subtype mRNAs in the prostate were measured by RT-PCR. The expression

of $\alpha 1A$ -adrenoceptor mRNA (Figure 3A) but not $\alpha 1D$ -adrenoceptor mRNA (Figure 3B) was reduced in the CAS+Vehicle group compared with the CON+Vehicle group. Conversely, the disease control group showed increased expression of $\alpha 1$ -adrenoceptor mRNA compared with the CON+Vehicle group. Expression of $\alpha 1A$ -adrenoceptor mRNA was significantly reduced in the BPH+L group compared with the disease control group. In the BPH+LTF group, expression levels of $\alpha 1A$ -adrenoceptor mRNA (0.52 ± 0.08) and $\alpha 1D$ -adrenoceptor mRNA (0.37 ± 0.15) were significantly lower than the disease control group and the BPH+L group.

The expression of $\alpha 1$ -adrenoceptor protein in prostate tissue

The expression changes in $\alpha 1$ -adrenoceptor proteins largely mirrored the mRNA responses, with increased expression in the disease control group compared with the

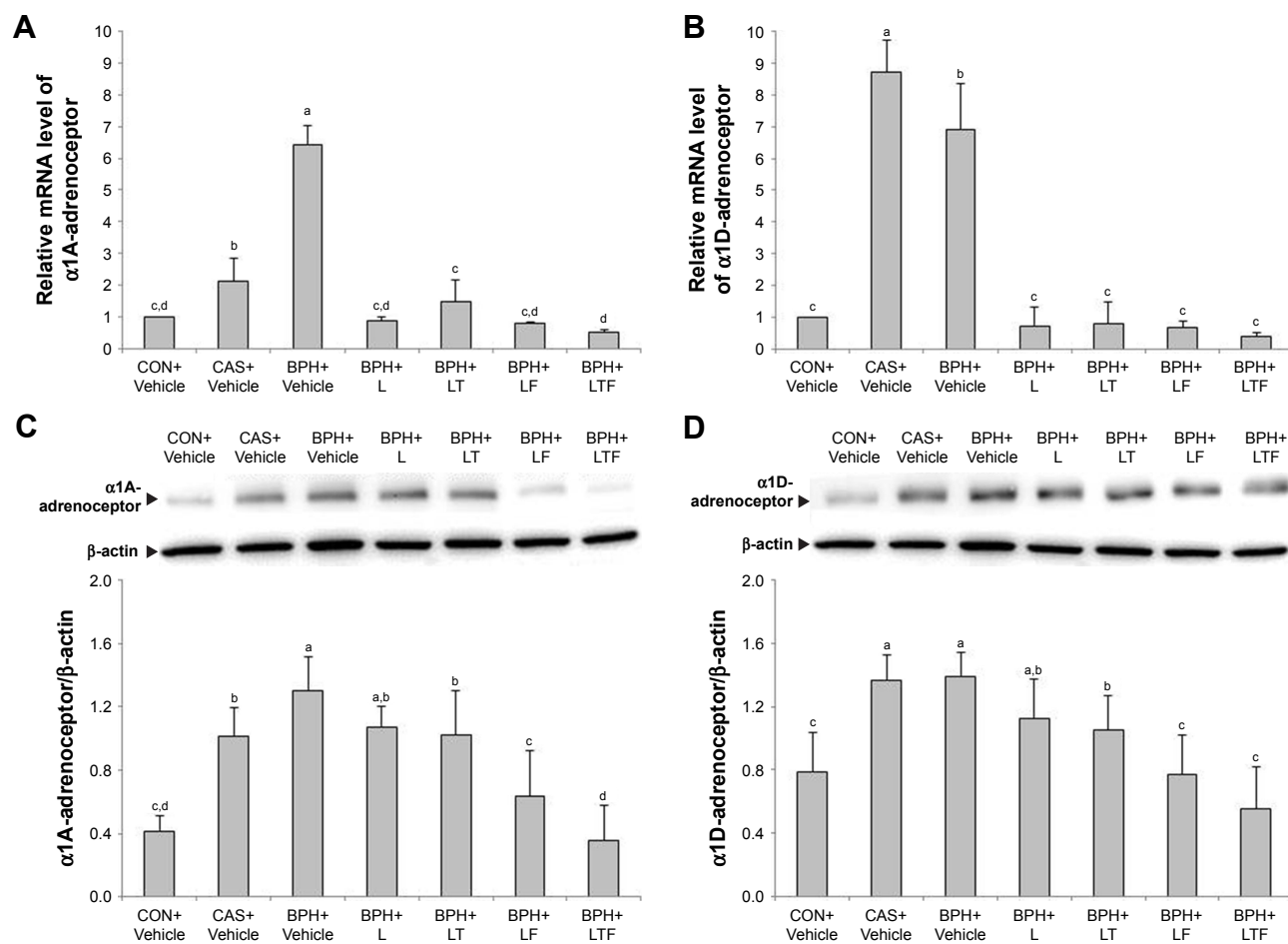


Figure 3 Effects of LDD175, tamsulosin and finasteride combinations on $\alpha 1$ -adrenoceptor subtype mRNAs and proteins expression in each treatment group as measured by real-time polymerase chain reaction and Western blotting.

Notes: (A) $\alpha 1A$ -adrenoceptor mRNA expression. (B) $\alpha 1D$ -adrenoceptor mRNA expression. (C) $\alpha 1A$ -adrenoceptor protein expression. (D) $\alpha 1D$ -adrenoceptor protein expression. Values with different superscript alphabets in the same row are significantly different ($P < 0.05$) by one-way analysis of variance and the Duncan's multiple range tests.

Abbreviations: BPH, benign prostatic hyperplasia; BPH+Vehicle, disease control; BPH+L, LDD175 (20 mg/kg); BPH+LT, LDD175 and tamsulosin (0.01 mg/kg); BPH+LF, LDD175 and finasteride (1 mg/kg); BPH+LTF, LDD175, tamsulosin, and finasteride; CAS+Vehicle, castration; CON+Vehicle, control.

CON+Vehicle group. The BPH+L group showed significantly reduced expression of $\alpha 1$ -adrenoceptor protein compared with the disease control group. In the BPH+LTF group, both $\alpha 1A$ -adrenoceptor protein expression (Figure 3C, 0.35 ± 0.22) and $\alpha 1D$ -adrenoceptor protein expression (Figure 3D, 0.56 ± 0.26) were significantly lower than the BPH+L group. Thus, LDD175, tamsulosin, and finasteride treatment was more effective than LDD175 alone.

Discussion

In the present study, oral administration of LDD175 with tamsulosin and finasteride significantly reduced the prostatic index, serum hormone levels, prostate epithelial thickness, prostate expression of $\alpha 1$ -adrenoceptors, and EST-induced elevation of IUP in a rat model of BPH.

The prostatic index is used as a clinical marker of BPH development⁵ and prostatic index is higher in animal models of BPH.²⁰ Finasteride and other agents commonly used to treat BPH clinically also decrease the prostatic index.²¹ The rat model established in this study exhibited an increased prostatic index compared with castrated rats, while LDD175 alone (BPH+L group) induced a reduction in prostatic index compared with the disease control group. Justulin et al²² reported that combined therapy decreased prostate volume to 33% of baseline after 30 days, while in the present study, combined therapy reduced prostate volume to 43% in BPH+LTF group. In addition, the BPH+LTF group demonstrated a dramatic reduction in both absolute prostate volume and prostatic index compared with the BPH+L group. These results indicate that combined administration of LDD175, tamsulosin, and finasteride attenuated prostatic enlargement induced by testosterone plus 17β -estradiol to a greater degree than LDD175 alone (or LDD175 with either tamsulosin or finasteride).

DHT is an important factor in BPH pathogenesis as it is the androgen primarily responsible for prostate growth.²³ DHT stimulates the transcription of growth factors that are mitogenic for prostate epithelial and stromal cells.⁷ Finasteride, a type II 5α -reductase inhibitor, that reduces epithelial cell size and the proliferative activity of DHT, is used for treating human BPH.²⁴ Surgical treatments, such as transurethral resection of the prostate, are performed most widely as the second option for patients who do not respond completely to combined finasteride plus tamsulosin therapy.²⁵ In the present study, LTF treatment reduced BPH-dependent DHT elevation to a greater extent than LDD175 alone. These results indicate that combined administration of LDD175, tamsulosin, and finasteride have additive or synergistic anti-proliferative effects, possibly by interfering

with androgen signaling. BPH involves the proliferation of prostate epithelial and stromal cells, resulting in increased prostate weight and volume.²⁶ The prostate is connected to the urethra by fascia and a series of ducts in rats.²⁷ When the prostate is sufficiently large, it can physically compress the urethra, resulting in partial or sometimes complete obstruction.²⁸ The present results showed the disease control group had increased IUP, while the combination of LDD175, tamsulosin, and finasteride had decreased IUP by relaxing and decreasing the prostatic smooth muscle. The disease control group showed marked epithelial hyperplasia compared with the CON+Vehicle group, which was only mild in BPH rats treated with LDD175 alone or a combination of LDD175, tamsulosin, and finasteride.

$\alpha 1$ -Adrenoceptor plays key point roles in the regulation of prostatic smooth muscle.²⁹ In BPH rat prostate weight and volume were increased, resulting in higher $\alpha 1$ -adrenoceptor than normal rats.³⁰ Because administration of finasteride reduced the volume of the prostate, it may decrease $\alpha 1$ -adrenoceptors. In this study, we observed increased $\alpha 1$ -adrenoceptor expression in the prostate tissue of BPH model rats, while both LDD175 alone (BPH+L group) and in combination (BPH+LTF group) reduced $\alpha 1$ -adrenoceptor expression compared to disease control group, with the combination therapy more effective. These findings indicate that combined administration of oral LDD175, tamsulosin, and finasteride may inhibit $\alpha 1$ -adrenoceptor activity in prostatic tissue. It is known that K_{ATP} channels are involved in the relaxation of prostatic smooth muscle.³¹ LDD175 is the most potent known activator of genitourinary smooth muscle BK_{Ca} channels as verified by dose-dependent relaxant activities on the urinary bladder of SD rats.³² In previous studies, intravenous injection of LDD175 significantly inhibited EST-induced IUP elevation in BPH model animals without a decrease in BP.²⁰

However, this *in vivo* study has limitations in that the experiment animal design had 7 groups. Many BPH studies previously had evidence that tamsulosin group, finasteride group, and tamsulosin + finasteride group reduced the increase of markers of BPH.^{5,26,33,34} Therefore, we designed the experimental group based on expectation about the pharmacological effect of LDD175 and the synergic effect of LDD175 with tamsulosin or/and finasteride in BPH rat model.

In summary, this study demonstrates that combined administration of LDD175 with tamsulosin and finasteride significantly reduces prostatic index, serum hormone levels, prostate epithelial thickness, prostate expression of $\alpha 1$ -adrenoceptors and EST-induced IUP elevation in a

BPH rat model. These results suggest that the combination of LDD175 with tamsulosin and finasteride is beneficial for lowering BPH-associated IUP and so may be an effective therapeutic alternative for patients who do not completely respond to selective α -1 blocker and 5- α reductase inhibitor therapy.

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Disclosure

The authors report no conflicts of interest in this work.

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