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FK143, a Novel Nonsteroidal Inhibitor of Steroid 5α-Reductase: (2) *In Vivo* Effects on Rat and Dog Prostates

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FK143 is a nonsteroidal new inhibitor of steroid 5α-reductase, an enzyme which converts testosterone into 5α-dihydrotestosterone (DHT). We studied *in vivo* effects of FK143 on rat and dog prostates. FK143 was orally administered to mature male rats for 14 days. At doses above 1 mg/kg, FK143 significantly reduced the wet weights of the ventral prostate and seminal vesicle, but showed no effects on those of the epididymis, testis, and adrenal. Growth of ventral prostate and seminal vesicle was induced by the subcutaneous injection of testosterone propionate (TP) in the castrated young rats and was reduced by FK143 administration at doses above 3.2 mg/kg, while growth induced by 5α-dihydrotestosterone propionate (DHTP) was not affected. FK143 had no binding affinity for the rat androgen receptor. FK143 showed neither estrogenic and antiestrogenic effects on the rat uterus nor androgenic effect on the rat prostate. Concentration of testosterone and DHT in the rat and dog prostates were measured by GC–MS, and administration of 10 mg/kg of FK143 significantly reduced the intraprostatic concentration of DHT. These results indicate that FK143 reduced the prostate growth by inhibiting 5α-reductase activities in the prostates.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) which is common among aged men, is characterized by progressive enlargement of the prostate gland and results in obstruction of urinary flow [1, 2]. Growth of the prostate gland is dependent on tissue androgen contents, and DHT is the most active agonist for the androgen receptor. Steroid 5α-reductase, which converts testosterone into DHT [3, 4], is highly active in the prostate gland and plays a crucial role in the prostate growth [5, 6]. It is expected that 5α-reductase inhibitors which inhibit DHT production but do not cause complete deprivation of all androgens will be useful in the therapy of DHT-dependent diseases such as BPH.

Various steroidal 5α-reductase inhibitors have been developed and experimental and clinical efficacies have been evaluated [7–10]. FK143, 4-[3-[3-[bis(4-isobutylphenyl)methylamino]benzoyl] - 1H - indol - 1 - yl]-butyric acid, is a newly synthesized compound which inhibits human and animal prostatic 5α-reductases. Being different from the preceding 5α-reductase inhibitors, FK143 has a nonsteroidal structure and inhibited 5α-reductases in a noncompetitive fashion [11].

In *in vitro* assay FK143 inhibited human prostatic 5α-reductase with an IC_{50} value almost the same as that of finasteride, which is a steroidal 5α-reductase inhibitor and already available for clinical use [12]. FK143 also inhibited rat, dog and monkey prostatic 5α-reductases with IC_{50} values similar to that of human enzyme.

In this report we studied the *in vivo* effects and the mode of action of FK143 in the rat and dog prostates. First FK143 was orally administered to the intact mature male rats and the inhibitory effects on the growth of prostate and other androgen-target organs were examined. Medical treatment of BPH to reduce

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Abbreviations: BPH, benign prostatic hyperplasia; CMA, chlormadinone acetate; DHT, 5α-dihydrotestosterone; DHTP, α-dihydrotestosterone propionate; GC–MS, gas chromatography–mass spectrophotometry; TBDMSC, *tert*-butyldimethylsilyl chloride; testosterone-d_3, 17β-hydroxyandrost-4-en-3-one-16, 16, 17-deuterium; TP, testosterone propionate.

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the prostate size includes different modes such as 5α-reductase inhibition, androgen blockade (anti-androgens) or medical castration (LH–RH analogues) [13, 14]. In order to exclude hormonal influences and define the mode of action, FK143 was also examined in the castrated young rats which were supplemented with exogenous androgens, testosterone or DHT. Estrogenic and antiestrogenic effects were examined in rat uteri. We measured rat intraprostatic contents of androgens to confirm the 5α-reductase inhibition by the drug. The accurate measurement of androgenic contents were achieved by using GC–MS. We also evaluated the effect of FK143 in the dog prostate, as dogs spontaneously undergo BPH and are commonly used as an animal model [15, 16]. *In vivo* effects of FK143 are discussed in comparison with finasteride.

MATERIALS AND METHODS

Chemicals

FK143 and DHT-d_3 were synthesized at the Exploratory Research Laboratories of Fujisawa Pharmaceutical Co., Ltd. TP, ethylenediaminetetraacetate disodium salt (EDTA) sodium molybdate (Na_2MoO_4), sodium laurylsulfate (SDS) and polyethylene glycol 400 (PEG 400) were purchased from Nacalai Tesque (Kyoto). Tris(hydroxymethyl)aminomethane (Tris), chlormadinone acetate (CMA), 17β-estradiol, α1-antitrypsin, leupeptin, activated charcoal and 17β-hydroxyandrost-4-en-3-one-16, 16, 17-deuterium (testosterone-d_3) were from Sigma (St Louis, MO, U.S.A.). Triamcinolone acetonide, dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were from Wako (Tokyo). DHTP was from Steraloids (Wilton). 17α-methyl-[^{3}H]mibolerone (specific activity 3219 GBq/mmol) and Aquasol-2 were from New England Nuclear (Boston). Dextran T-70 and LH 20 were from Pharmacia (Uppsala). Lipidex 5000 was from Packard (Groningen). Sep pak C18 cartridge was from Waters (Milford). *Tert*-butyldimethylsilyl chloride (TBDMSC) was from Aldrich Chemical (Milwaukee).

Animals

Male and female Wistar rats and male Sprague–Dawley rats were supplied by Nihon Clea (Tokyo). Mature male Beagle dogs, weighing approx. 10 kg, were supplied by Japan E. D. M. Inc.(Tokyo). All animals were fed commercially available chow and water was supplied *ad libitum*. They were housed in temperature-controlled rooms with lights on between 08:00 h and 20:00 h.

Effects in mature male rats

Mature male Wistar rats were domesticated for a week, and oral administration was started at 9 weeks old with 5 ml/kg of drug suspension once daily for 14 consecutive days [17]. Drugs were suspended in 0.5% methylcellulose solution. Rats were sacrificed by CO_2 gas on the day after the last dosing and weighed. The following organs were removed and weighed: ventral prostates, seminal vesicles, testes, epididymides, and adrenals. Organ weights were recalculated (mg/100 g body weight) by dividing the values by the body weights.

Effects in castrated young rats

Four-week-old prepubertal male Wistar rats were anesthetized by pentobarbital and castrated. From the incised abdomen vas deferens, testicular artery and vein were fastened and testes were cut out [18]. After 3 days interval, oral administration of 5 ml/kg of drug suspension was started once daily for 5 consecutive days. Drugs were suspended in 0.5% methylcellulose solution. Three hundred μg/kg of TP or 900 μg/kg of DHTP in sesame oil were subcutaneously injected to the rats at the same time of drug administration. Rats were sacrificed by CO_2 gas about 6 h after the last dosing, and ventral prostates and seminal vesicles were removed and weighed.

Binding assay for the androgen receptor of rat prostatic cytosol

Binding of the drug to cytoplasmic androgen receptor of rat prostate was determined by standard Dextran-coated charcoal adsorption techniques [19]. Nine-week-old male Sprague–Dawley rats were castrated and sacrificed by CO_2 gas 24 h after the castration. Ventral prostates were removed and immediately washed with ice cold buffer (10 mM Tris–HCl buffer, pH 7.4, 1 mM EDTA, 10 mM sodium molybdate, 10% (v/v) glycerol, 5 mM dithiothreitol, 25 μg/ml of α1-antitrypsin and 25 μg/ml of leupeptin). All the following procedures were carried out at 4°C, Fresh prostate was homogenized in the buffer (0.5 ml/prostate) with a Polytron homogenizer. The homogenate was centrifuged at 108,000 *g* for 1 h in a Hitachi ultracentrifuge (SCP55H) using a RP55T rotor. The resulting supernatant was used as the source of cytosolic androgen receptor and stored −80°C until use.

Ten μl of samples, FK143 or CMA dissolved in DMSO, or DMSO control were incubated with 90 μl of rat prostatic cytosol and 100 μl of 17α-methyl-[^{3}H]mibolerone (diluted with assay buffer to the final concentration of 5 nM) at 4°C overnight. Assay buffer contained 5 μM triamcinolone acetonide to inhibit binding of radioactive ligand to progesterone and glucocorticoid receptors. Nonspecific binding was determined by carrying out parallel incubations containing 10 nM nonradioactive testosterone. Bound and free ligand was separated by adding 200 μl of Dextran-coated charcoal suspension (containing 0.5% activated charcoal and 0.05% Dextran T-70 in the buffer). After further incubation for 10 min at 4°C, tubes were

centrifuged at 1500 *g* for 5 min. 100 μl of the supernate was suspended in 10 ml of Aquasol-2 and counted by the scintillation counter.

Estrogenic and antiestrogenic effect in rat uteri

Three-week-old female Wistar rats were orally administered 5 ml/kg of drug suspension for 3 consecutive days in the presence or absence of s.c. injection of 17β-estradiol [20]. Drugs were suspended in 0.5% methylcellulose solution. Rats were sacrificed by CO_2 gas on the next day of the last dosing, and the uteri were removed and weighed.

Androgenic effect in rat prostate

Four-week-old prepubertal male Wistar rats were anesthetized by pentobarbital and castrated. After 3 days interval, oral administration of 5 ml/kg of FK143 suspension was started once daily for 5 consecutive days. Drugs were suspended in 0.5% methylcellulose solution. Rats were sacrificed by CO_2 gas about 6 h after the last dosing, and ventral prostates and seminal vesicles were removed and weighed.

Testosterone and DHT determination

Intraprostatic concentration of testosterone and DHT were measured according to the previously described methods [21]. Mature male Beagle dogs were treated 30 min after the meal with oral administration of the drug 4 times at the 12 h intervals. The drug was dissolved in PEG 400 and packed in the gelatin capsule. Six hours after the last dosing, dogs were anesthetized by the pentobarbital and killed by bleeding from the femoral artery. Whole prostate glands were removed, quickly frozen in liquid nitrogen, and stored at −80°C. Just before the measurement the tissues were thawed, dissected free of their capsules and fluids, washed with saline, weighed, minced with scissors and homogenized in water with a polytron homogenizer on ice. All the devices and glass containers were washed with ethanol before use. The protein concentration of the homogenate was determined. The homogenate was added with SDS and NaOH in the final concentration of 5% and 0.5 N, respectively, and incubated at 40°C for 2 h. 600 mg of tissue suspension was added, with 500 pg of testosterone-d_3 and DHT-d_3 as the internal standards. Testosterone and DHT were extracted 3 times with 6 ml of diethyl ether and evaporated. The residue was dissolved in the elution solvent (methanol:water:chloroform = 9:1:2) and testosterone and DHT were eluted through Lipidex 5000 resin. The eluate was evaporated, dissolved in methanol, 3 times the volume of water was added, applied on Sep pak C18 cartridges, consecutively washed with 10, 25 and 50% methanol and eluted with 100% methanol. One hundred % methanol eluate was evaporated and the residue was treated with 50 μl of TBDMCS/imidazole/DMF (1:1:6) at 50°C for 1 h and evaporated. The residue was dissolved and eluted through the LH 20 column (5φ × 20 mm) in the solvent (hexane:chloroform:methanol = 10:10:1), evaporated, dissolved in 50 μl of ethyl acetate and injected in GC–MS. Testosterone and DHT solutions were used as the standards. GC–MS conditions were as follows: Hewlett Packard 5890 as GC, Ultra 1 (HP) 25 m × 0.32 mm × 0.17 μm as the column, VG ZAB-SE as MS.

Mature male Wistar rats were orally administered once with the drug suspended in 0.5% methylcellulose solution in the concentration of 5 ml/kg. Twelve hours after the administration, rats were weighed, anesthetized with pentobarbital, and killed by bleeding. The ventral prostates were dissected free of their capsules, weighed, washed with saline and stored at −80°C. The extraction and measurement of testosterone and DHT were carried out as the same methods described above.

In order to estimate the long-term effect of the drug, mature male Wistar rats were orally administered with the drug suspension once daily for 14 consecutive days. On the next day of the last dosing rats were weighed, sacrificed and ventral prostates were removed. The following procedure was the same as above.

Protein determination

Protein concentrations were determined using a Bio-Rad Protein Assay Reagent using bovine serum albumin as the standard.

Statistics

Statistical analyses to assess comparisons between multiple treatment groups were based on the method of Dunnett's *t*-test using a Stat View for a Macintosh computer. Comparisons between two treatment groups were based on the method of Student's *t*-test.

RESULTS

Effect of FK143 on rat organ weights

Inhibitory effects of FK143 on organ weights were estimated in the intact rats. Mature male Wistar rats were orally administered with FK143 for 14 consecutive days at doses from 0.32 to 32 mg/kg. Figure 1 shows that FK143 significantly and dose-dependently reduced the weights of the ventral prostate and seminal vesicle at doses above 1 mg/kg, and inhibitory ratios to the ventral prostate and seminal vesicle at 32 mg/kg were 30.6 and 56.0%, respectively. Administration of FK143 showed no difference in body weight gain (Table 1). Testis, epididymis, and adrenal were also weighed (Table 1). FK143 showed almost no effects on the weights of these organs.

Effect of FK143 on castrated young rats

In order to exclude the possibility that FK143 may act through endocrine glands such as pituitary or testis, effects of FK143 were estimated in the castrated rats. One week after the castration the weights

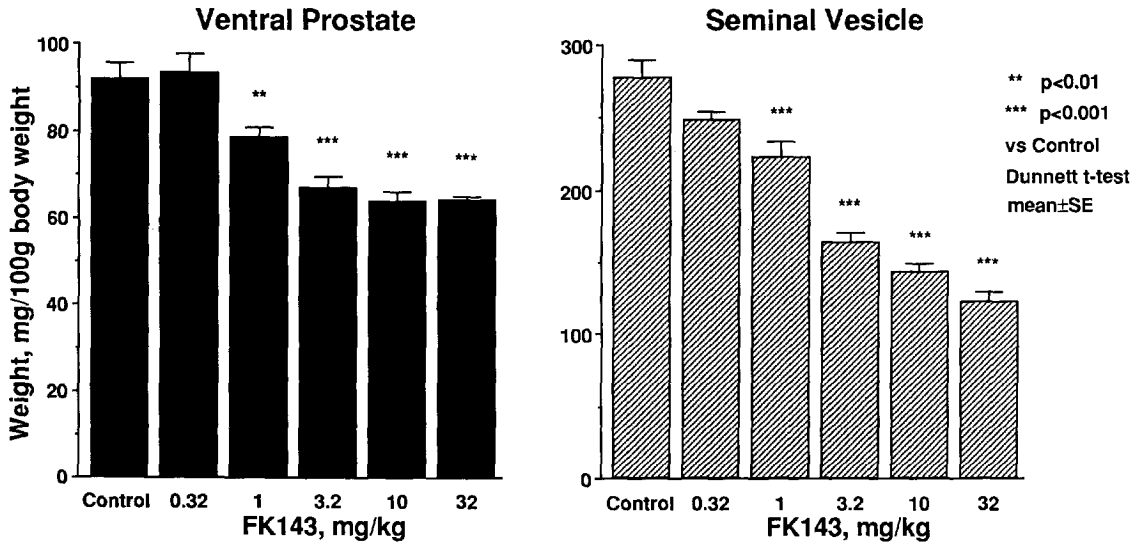

Fig. 1. Effects of FK143 on organ weights of mature male rats. Rats were orally administered with drug suspension for 14 days. On the next day of the last dosing, organs were removed and weighed. These results are the average of 3 experiments. Values represent mean ± SE. Animal numbers are as follows: control, 20; 0.32 mg/kg, 16; 1 mg/kg, 21; 3.2 mg/kg, 19; 10 mg/kg, 20; 32 mg/kg, 13.

of ventral prostate and seminal vesicle were markedly reduced, while the injection of 300 μg/kg of TP or 900 mg/kg of DHTP restored those weights (Figs 2 and 3).

As shown in Fig. 2, FK143 was administered to the castrated rats receiving TP and dose-dependently reduced the weights of the ventral prostate and seminal vesicle. Statistically significant reduction was observed at doses above 3.2 mg/kg. Figure 3 shows that chlormadinone acetate (CMA), an antiandrogenic agent, dose-dependently reduced the DHTP-induced growth of the ventral prostate and seminal vesicle. On the other hand, FK143 showed no reduction in the weights of the ventral prostate and seminal vesicle at 32 mg/kg or less.

Binding assay for rat androgen receptor

Binding assay for the androgen receptor was carried out by using rat prostatic cytosol as the receptor source. DHT showed dose-dependent receptor binding and completely displaced the binding of 17α-methyl-[^{3}H]mibolerone at 10^{-7} M (Fig. 4). CMA showed almost the same affinity for the rat androgen receptor as DHT. FK143 showed no displacement of the ligand at 10^{-5} M or less.

Evaluation of hormonal effects of FK143

The androgenic effect of FK143 was determined by evaluating growth stimulating effects on the ventral prostate and seminal vesicle in the castrated young rats (Fig. 5). While s.c. injection of TP induced significant growth of the ventral prostate and seminal vesicle, FK143 induced no increase in the weights of the ventral prostate and seminal vesicle at 100 mg/kg or less.

Estrogenic and antiestrogenic effects of FK143 were determined by evaluating growth stimulating and growth inhibiting effects on the rat uteri (Fig. 6). Figure 6(A) shows that the rat uterine growth was significantly induced by the injection of 17β-estradiol at doses above 0.1 mg/kg. FK143 did not promote uterine growth as seen in 17β-estradiol injected rats.

Table 1. Effects of FK143 on organ weights of mature rats

Drugs (mg/kg)	n	Body weight gain (g)	Ventral prostate (mg/100 g b.w.)	Seminal vesicle (mg/100 g b.w.)	Testis (mg/100 g b.w.)	Epididymis (mg/100 g b.w.)	Adrenal (mg/100 g b.w.)
Control	(20)	43.8 ± 4.3	92.2 ± 3.6	277.2 ± 11.9	905.2 ± 15.2	255.5 ± 7.5	15.2 ± 0.6
FK143							
0.32	(16)	43.4 ± 3.4	93.5 ± 4.3	248.4 ± 6.2	916.1 ± 11.1	250.6 ± 7.5	16.5 ± 0.5
1	(21)	41.7 ± 2.9	78.5 ± 2.4**	222.3 ± 11.2***	874.7 ± 17.6	251.1 ± 8.6	16.2 ± 0.6
3.2	(19)	41.1 ± 3.3	67.0 ± 2.5***	163.1 ± 7.5***	913.2 ± 10.4	264.9 ± 6.8	17.3 ± 0.5*
10	(20)	38.5 ± 4.9	63.9 ± 2.4***	142.4 ± 6.0***	939.5 ± 15.0	269.0 ± 7.7	17.0 ± 0.6
32	(13)	48.3 ± 3.9	64.0 ± 1.3***	122.0 ± 7.1***	943.9 ± 17.5	249.7 ± 10.6	17.0 ± 0.6

Values represent mean ± SE. Statistical analyses were based on the method of Dunnett t-test.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs Control.

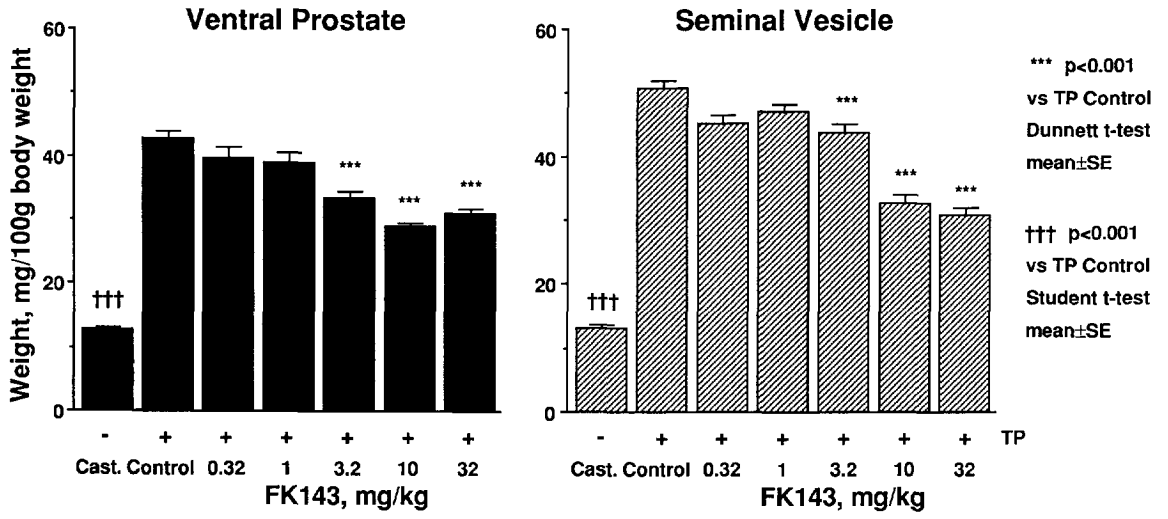

Fig. 2. Effects of FK143 on organ weights of castrated young rats (TP-treated). Prepubertal male rats were castrated, subcutaneously injected with 300 μg/kg of TP (except castrated control), and orally administered with drug suspension for 5 days. These results are the average of 5 experiments. Values represent mean ± SE of 25 animals except the treatment groups with 0.32 mg/kg of FK143 (10 animals) and 32 mg/kg of FK143 (5 animals).

Moreover, FK143 did not suppress 17β-estradiol induced uterine growth [Fig. 6(B)].

Testosterone and DHT determination in rat and dog prostate

The direct effects of FK143 on the androgen metabolism in the rat and dog prostates were estimated by using GC–MS. Intraprostatic testosterone was almost converted into DHT (Figs 7 and 8). Twelve hours after the single administration of FK143 to the mature male rats, DHT concentration in the ventral prostate was significantly and dose-dependently reduced at doses above 3.2 mg/kg, while testosterone concentrations were conversely increased (Fig. 7). Long-term effects of FK143 on the testosterone and DHT concentrations were also estimated in the rat prostates. Fourteen days administration of FK143 dose-dependently reduced the DHT concentration in the rat ventral prostate, and slightly increased the testosterone concentration (Fig. 7). Mature male Beagle dogs were administered 4 times at 12 h intervals with 10 mg/kg of FK143 (Fig. 8). FK143 significantly reduced DHT concentration and significantly increased testosterone concentration in the dog prostate.

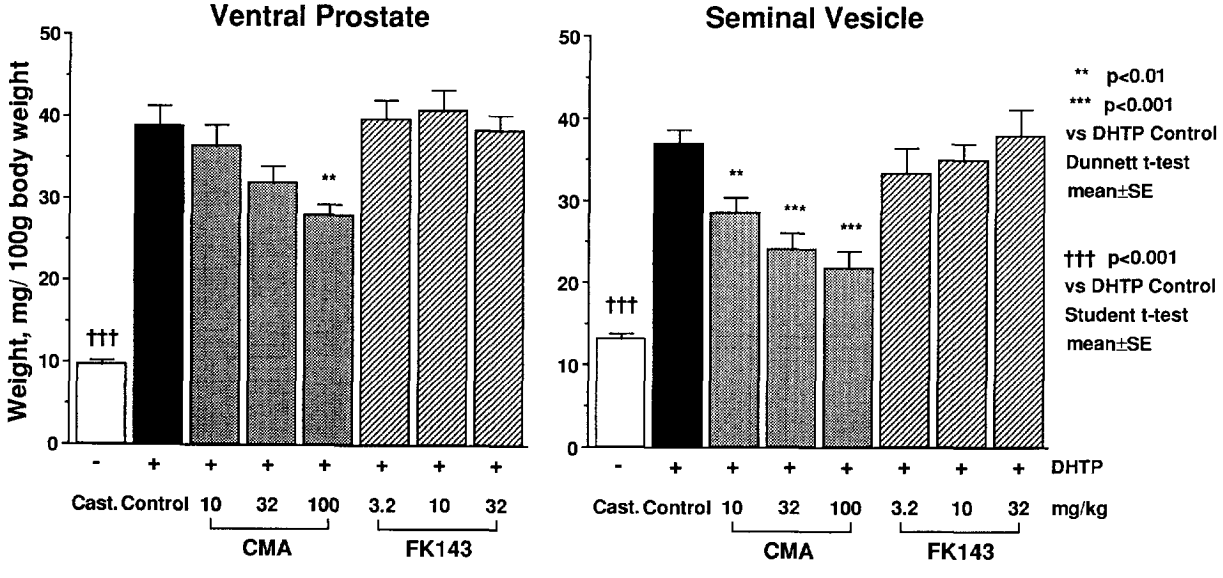

Fig. 3. Effects of drugs on organ weights of castrated young rats (DHTP-treated). Prepubertal male rats were castrated, subcutaneously injected with 900 μg/kg of DHTP (except castrated control), and orally administered with drug suspension for 5 days. Values represent mean ± SE of 10 animals except 10 mg/kg of chlormadinone acetate (CMA) treated group (9 animals).

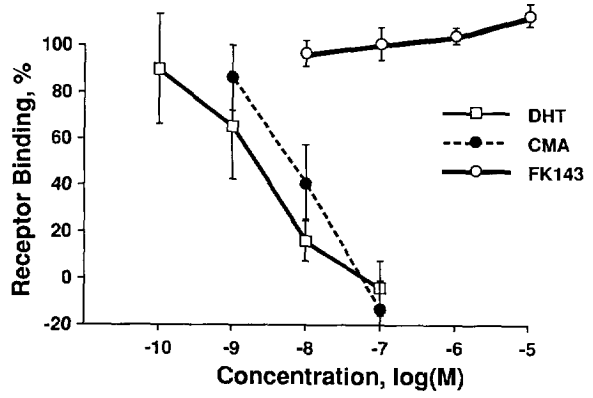

Fig. 4. Binding affinity for the rat prostatic androgen receptor. Binding affinities of the increasing concentrations of the drugs were measured by displacement of 5 nM 17α-methyl-[^{3}H]mibolerone from cytosolic androgen receptor of rat prostate. Values represent mean ± SE of 4 (DHT) or 2 (chlormadinone acetate, FK143) experiments.

DISCUSSION

Human prostate growth is an age-related, androgen-dependent process, and 5α-reductase plays the crucial role [5, 6] by mediating DHT which accounts for the greater part of intraprostatic androgens [22] and is physiologically most active for the prostate growth. As there are many similarities between human and rat prostate growth, rat prostate is commonly used as the growth model, although histological and biochemical differences still exist. Atrophic effects on rat prostate have been evaluated by using several steroidal 5α-reductase inhibitors [17, 23, 24]. Recently rat 5α-reductases have been isolated and shown to be expressed in the ventral prostate [25, 26]. They showed similarities to human enzymes in amino acid sequences (approx. 60% homology) and activities, but different pharmacological responses were also shown [27]. In the preceding paper we investigated *in vitro* effects of FK143 on human and animal prostatic 5α-reductases, and almost equivalent IC_{50} values were obtained in human, dog, and rat 5α-reductases [11]. In this report we demonstrate *in vivo* effects and the mode of action of FK143 in animal prostates.

As shown in Fig. 1, inhibitory effects of FK143 on the organ growth were evaluated in mature male rats. Treatment with FK143 significantly and dose-dependently reduced weights of the ventral prostate and seminal vesicle. The magnitude of the reduction of these organ weights were almost equal with those by other steroidal 5α-reductase inhibitors [23, 24]. These results may be ascribed to the blockade of DHT formation in these organs by 5α-reductase inhibition. FK143 showed no effects on rat body weight gain (Table 2). FK143 failed to reduce the weight of epididymis, one of the androgen-target organs [28] and contains 5α-reductase. This may result from the extremely high activity of 5α-reductase in this organ. Weights of other androgen-related organs such as testis or adrenal were not affected by FK143.

Besides 5α-reductase inhibitors, several drugs were known to reduce prostate growth in the different modes. LH–RH analogues [29, 30] reduce prostate weight through the hypothalamic–pituitary–testicular system. We evaluated the effect of FK143 in the castrated rats to exclude the hormonal influences from the inhibitory effect of FK143. One week after castration the weights of the ventral prostate and seminal vesicle were markedly reduced, and exogenous injection of TP and DHTP efficiently restored these organ weights (Figs 2 and 3). FK143 inhibited TP-induced growth of the ventral prostate and seminal vesicle in the castrated rats in the same magnitudes as those in the intact mature rats (Fig. 1). This result

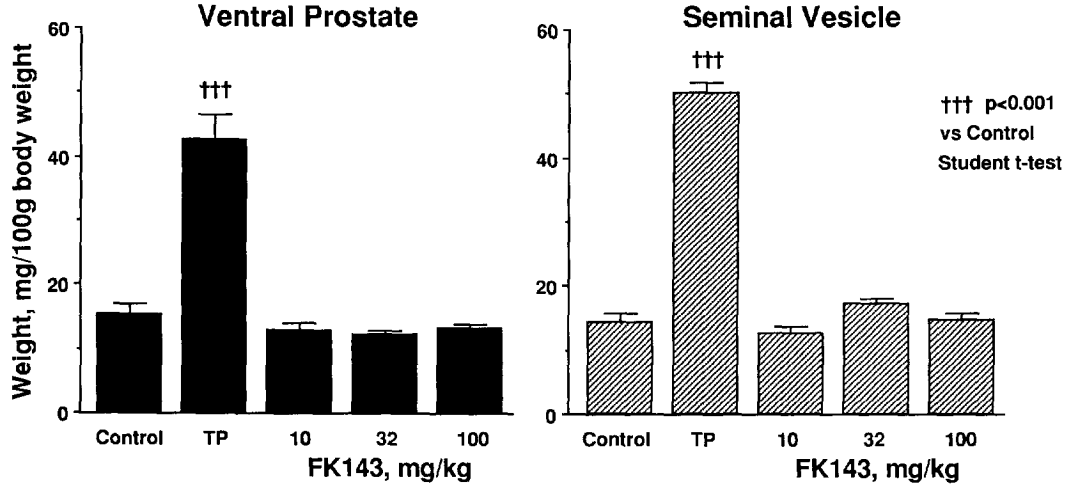

Fig. 5. Androgenic effects of FK143 on organ weights of castrated young rats. Castrated young rats were subcutaneously injected with 300 μg/kg of TP, or orally administered with FK143 for 5 days. Values represent mean ± SE of 5 animals.

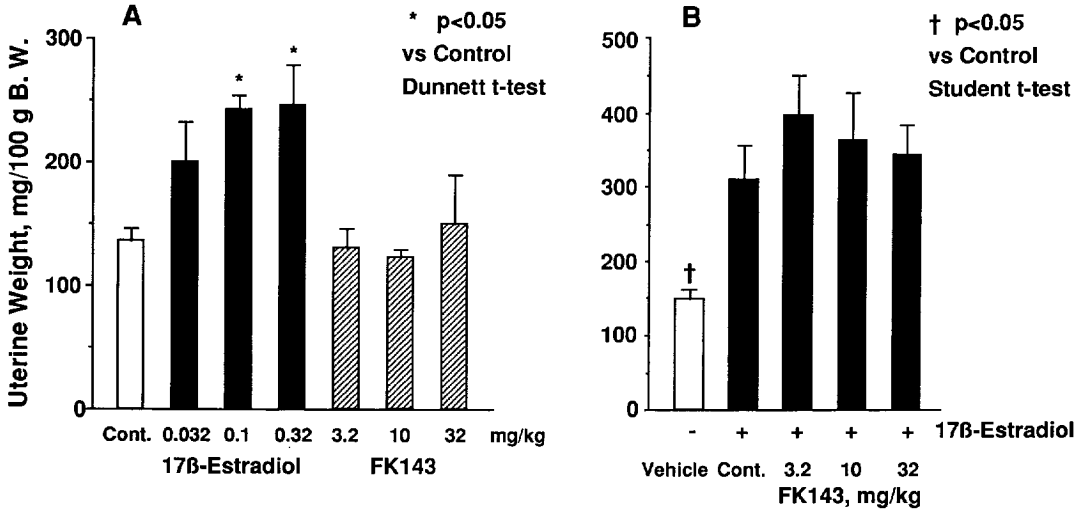

Fig. 6. Estrogenic and antiestrogenic effects of FK143 on rat uterine weight. (A) Female rats were subcutaneously injected with 17β-estradiol, or orally administered with FK143 for 3 days. (B) Female rats were subcutaneously injected with 0.1 mg/kg of 17β-estradiol and orally administered with FK143 at the same time for 3 days. Values represent mean ± SE of 5 animals.

excludes the participation of the central nervous system in the inhibitory effect of FK143 in rat prostate growth. On the other hand, antiandrogens suppress animal prostate growth induced by DHT-inducible androgens [31, 32]. Chlormadinone acetate (CMA), a clinically used antiandrogen, dose-dependently displaced the binding of the ligand to androgen receptor, and inhibited DHTP-induced growth of the ventral prostate and seminal vesicle (Fig. 3). FK143 showed neither affinity for androgen receptor nor inhibition of DHTP-induced organ growth (Figs 3 and 4). These results exclude the participation of antiandrogenic effect on the atrophic effect of FK143. Androgenic effect was also evaluated by the growth stimulation of the ventral prostate in the castrated rat, and FK143 showed no androgenic effect (Fig. 5).

The possible role for estrogen in the pathogenesis of BPH has been suggested [13]. The ratio of plasma estrogen to testosterone is increased in aging males with BPH, and estradiol levels as well as estrogen receptor concentration are in greater abundance in the stroma [33]. The evidence indirectly suggests that estradiol may have relevance to the stromal overgrowth in BPH. Estrogen deprivation by aromatase inhibitor suppressed the estrogen related stimulation of fibromuscular stroma of the dog prostate [34]. Estrogenic effect was evaluated by the growth stimulation and growth suppression of the rat uterus, and FK143 showed

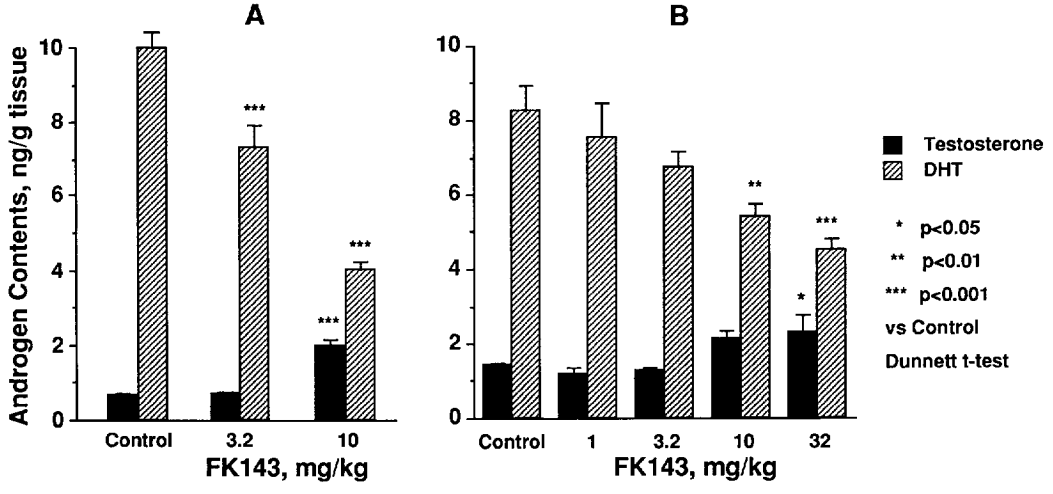

Fig. 7. Effects of FK143 on androgen contents in the rat prostates. (A) Mature male rats were orally administered with FK143 once. Animal numbers are as follows: control, 7; 3.2 mg/kg, 5; 10 mg/kg, 5. (B) Mature male rats were orally administered with FK143 once daily for 14 days. Animal numbers are as follows: control, 5; 1 mg/kg, 5; 3.2 mg/kg, 5; 10 mg/kg, 4; 32 mg/kg, 5. Testosterone and DHT contents in the ventral prostates were determined by GC–MS. Values represent mean ± SE.

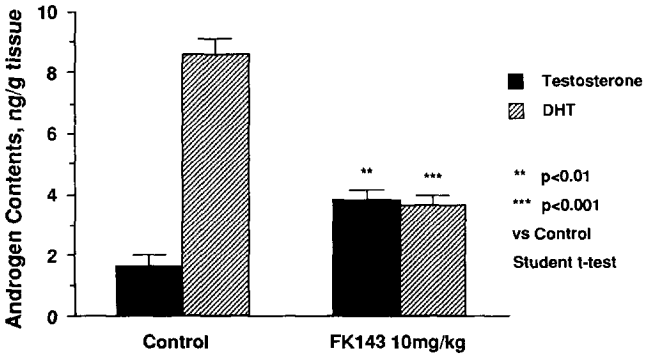

Fig. 8. Effect of FK143 on androgen contents in the dog prostates. Mature male dogs were administered with FK143 after the meal 4 times at the 12 h intervals. Testosterone and DHT contents in the whole prostates were determined by GC–MS. Values represent mean ± SE of 4 animals.

neither estrogenic nor antiestrogenic effects (Fig. 6). These results exclude the participation of an antiestrogenic effect on the atrophic effect of FK143.

The direct evidence of 5α-reductase inhibition by FK143 was demonstrated by measurement of intraprostatic androgen concentrations. Radioimmunoassay is commonly used to determine the concentration of serum testosterone and DHT [35]. However, commercially available radioimmunoassay kits are unsatisfactory, because testosterone antiserum cross-reacts with DHT and DHT concentration is much higher than that of testosterone in the prostate. Accurate determination of testosterone and DHT was achieved by GC–MS by using deuterium-labeled testosterone and DHT as the internal standards [21]. The greater part of the intraprostatic testosterone was converted into DHT in the rat prostate (Fig. 7). A single administration of FK143 significantly reduced DHT concentration and conversely increased testosterone concentration at the dose of 10 mg/kg. The long-term effect of FK143 was also evaluated in the rats administered for 14 days, in the same schedule as Fig. 1. FK143 dose-dependently reduced DHT concentration and significantly reduced at doses above 10 mg/kg, although the magnitude of the reduction is somewhat weaker than that of the single administration. Since rat 5α-reductase expression is regulated by its own biosynthetic androgens [36], it is possible that long-term treatment of FK143 changed the ratio of testosterone to DHT in the rat prostate and might induce 5α-reductase activity.

Finasteride, a steroidal 5α-reductase inhibitor, has been introduced to clinical use for BPH. Although finasteride undoubtedly reduced the prostate size, the involution rate is not complete compared to the castrated animals [24]. One of the possible reasons of the incomplete efficacy is the accumulation of testosterone in the prostate gland, which is commonly observed in the animals and human that received finasteride [22, 37–39]. Accumulated substrate may theoretically counteract the competitive inhibitors on the active site of the enzyme and reduce the inhibitory effects. Accumulation of testosterone coupled with the reduction of DHT is also observed in the prostate of rats and dogs that received FK143 (Figs 7 and 8). On the other hand, we have demonstrated that FK143 inhibited human and rat 5α-reductase in a noncompetitive fashion, and the inhibitory action of FK143 on 5α-reductase was not affected by testosterone concentration while the inhibitory action of finasteride was attenuated by the increasing concentration of testosterone *in vitro* [11]. These observations suggest that noncompetitive inhibitors of 5α-reductase could avoid the competitive effect of accumulated testosterone on the enzyme. However, FK143 did not completely reduce prostate weight to the castrated level even at 32 mg/kg (Fig. 2) in spite of its advantageous characteristic. One of the possible explanations is that the intraprostatic DHT concentration is not reduced to the castrated level. Another is that increased concentration of testosterone partly compensated for reduced DHT activity in the prostate growth, because a high concentration of testosterone itself interacts with androgen receptor [40]. The latter characteristic is common to the 5α-reductase inhibitors described to date.

Dogs are considered to be the most reliable animal model to evaluate the medical efficacy of drugs for BPH. Like human prostate, dog prostate size increases in an age-related, androgen-dependent manner and BPH spontaneously occurs. The atrophic effect of 5α-reductase inhibitor in the dog prostate has been reported [41, 42]. We determined the short-term effect of FK143 on DHT content in the dog prostate. Dog prostate contained high concentrations of DHT and 4 times administration of 10 mg/kg of FK143 significantly reduced DHT concentration. Considering the results in the rat, long-term effect of FK143 on dog prostate size and DHT content should be further evaluated.

In conclusion, FK143 inhibited rat prostate growth and reduced DHT content in rat and dog prostates. FK143 showed no hormonal effects such as androgen, antiandrogen, estrogen and antiestrogen. The atrophic effect of FK143 on rat prostate is defined as 5α-reductase inhibition. These results suggest that FK143 would be a good candidate for use in men with BPH.

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