Pergamon

0960-0760(94)00187-1

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 4, pp. 357–363, 1995

0960-0760/95 $9.50 + 0.00

FK143, a Novel Nonsteroidal Inhibitor of Steroid 5α-Reductase: (1) *In Vitro* Effects on Human and Animal Prostatic Enzymes

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Steroid 5α-reductase is an enzyme which converts testosterone into 5α-dihydrotestosterone (DHT) and is implicated in the pathogenesis of benign prostatic hyperplasia (BPH) in men. We studied *in vitro* effects of FK143, a nonsteroidal new compound, on 5α-reductase in human and animal prostates. Prostates were obtained from Wistar rats, Beagle dogs, and Cynomolgus monkeys as well as prostatic tissue from BPH patients obtained by the prostatectomy. Nuclear membrane fraction of prostates showed pH dependent 5α-reductase activities, and inhibitory effects of drugs were assayed at pH 6.5. FK143 inhibited human prostatic 5α-reductase in a dose-dependent manner with an IC_{50} of 1.9 nM and also inhibited animal 5α-reductases with similar IC_{50} values. FK143 inhibited human and rat 5α-reductases in a noncompetitive fashion while finasteride, a steroidal 5α-reductase inhibitor, showed competitive inhibition. The affinities of FK143 for the human 5α-reductase is constant at pH 5 and 6.5. No inhibitory effects were shown to other oxidoreductases. These results indicate that FK143 is a new type of potent and selective 5α-reductase inhibitor.

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INTRODUCTION

Steroid 5α-reductase is a membrane bound, NADPH dependent enzyme which converts testosterone into DHT [1, 2] and has been located in some of the androgen-target tissues, such as prostate, seminal vesicle, epididymis, liver, and skin. DHT is supposed to have some roles in pathological conditions such as BPH, acne, female hirsutism, prostatic cancer, and male pattern baldness [3].

BPH is characterized by progressive enlargement of the prostate gland [4, 5]. It is supposed that growth of the prostate gland is dependent on tissue androgen contents [6], and DHT is the most active agonist for the androgen receptor [7] although testosterone also mediates the androgenic action in the tissues. According to this supposition antiandrogens and LH-RH analogues have been used for BPH until now. However, the major problem of these drugs is the severe side effects caused by the complete deprivation of androgens [8]. On the other hand, the essential role of 5α-reductase in prostate growth has been revealed by the discovery of 5α-reductase deficiency in male pseudohermaphroditism patients who had atrophic prostate glands despite normal testosterone contents [9, 10]. Various steroidal compounds have been developed as 5α-reductase inhibitors for the medical therapy of BPH [3, 11]. Finasteride [12], one of these steroidal 5α-reductase inhibitors, is already available for clinical use and the evaluation of therapeutic efficacy to relieve the BPH symptoms is now under study [13–15].

FK143, 4-[3-[3-[bis(4-isobutylphenyl)methylamino]benzoyl]-1H-indol-1-yl]butyric acid (Fig. 1), is a newly synthesized nonsteroidal compound to optimally inhibit human 5α-reductase activity. In this report we studied the inhibitory potency of FK143 on prostatic 5α-reductases from human and other animal species, rat, dog and monkey. We also examined its nature of inhibition compared with that of finasteride.

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Abbreviations: BPH, benign prostatic hyperplasia; DHT, 5α-dihydrotestosterone; LH-RH, luteinizing hormone-releasing hormone.

Received 28 Jun. 1994; accepted 28 Oct. 1994.

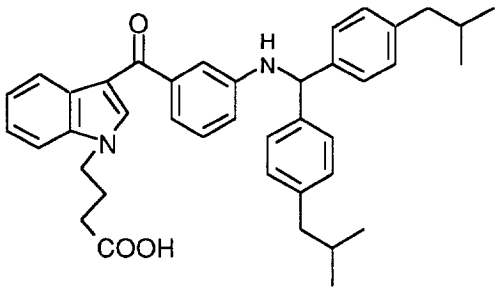

Fig. 1. Structure of FK143.

MATERIALS AND METHODS

Chemicals

FK143 and finasteride were synthesized at the Exploratory Research Laboratories of Fujisawa Pharmaceutical Co., Ltd. [1,2,6,7-^{3}H]testosterone, [1,2,4,5,6,7-^{3}H]DHT, and Aquasol-2 were purchased from New England Nuclear (Boston). Kieselgel 60 F_{254} and the aluminium oxide coated TLC plate (60 F_{254} neutral type E) were purchased from Merck (Darmstadt). Testosterone, primuline, and dithiothreitol (DTT) were from Nacalai Tesque (Kyoto). Ethyl acetate was from Wako (Osaka). DHT and tris(hydroxymethyl)aminomethane (Tris) were from Sigma (St Louis, MO, U.S.A.). Nicotinamide adenine dinucleotide phosphate reduced form (NADPH) was from Kohjin (Tokyo). Dye reagent concentrate was from Bio-Rad (Hercules).

Animals

Mature male Wistar rats, approx. 10–20 weeks old, were supplied by Nihon Clea (Tokyo). Mature male Beagle dogs, weighing approx. 10 kg, were supplied by Japan Laboratory Animals, Inc. (Tokyo). Mature male Cynomolgus monkeys, weighing approx. 4 kg, were supplied by Hazleton Research Products, Inc. (Denver). 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.

Preparation of prostatic enzyme

Animals were anesthetized by injecting pentobarbital and killed by the bleeding from aorta or femoral artery. The ventral prostates of rats were dissected free of their capsules, washed with saline, and stored at −80°C. The whole prostates of dogs and monkeys were also collected as above. Human prostatic tissues from BPH patients who received transurethral prostatectomy were kindly provided by Dr M. Tachibana at Hospital of Keio University, and stored at −80°C, Prostatic enzyme fractions were prepared as previously described [15]. Frozen tissues were thawed on ice and minced with scissors. Unless specified, all the following procedures were carried out at 4°C. The tissues were homogenized with Polytron homogenizer in 3–4 tissue volumes of medium A (0.32 M sucrose, 0.1 mM DTT, and 20 mM sodium phosphate buffer pH 6.5). The homogenates were centrifuged at 1500 *g* for 20 min, and the nuclear membrane fractions were precipitated. The pellets were resuspended in medium A and filtered with gauze. The suspension (3–10 mg/ml) was stored at −80°C until use.

Five α-reductase assay

Five α-reductase activities were assayed as previously described [16]. The reaction mixture contained in a final volume of 200 μl: 1 mM DTT, sodium or potassium phosphate buffers, 0.1–2 mM NADPH, 2 nM [1,2,6,7-^{3}H]testosterone, and the prostatic enzyme fraction. The amount of prostatic enzyme fractions were settled to adjust the rate of conversion of testosterone into DHT to around 30% at pH 6.5. Testosterone concentrations were adjusted to 2–108 nM by adding cold testosterone. The reaction mixture in duplicate were started by adding the enzyme fraction, incubated at 37°C for 25–60 min, and stopped by mixing with 200–300 μl of ethyl acetate containing cold 500 μg/ml of testosterone and 300 μg/ml of DHT as UV markers (245 nm for testosterone, 305 nm for DHT). Fifty μl of ethyl acetate was spotted on the Kieselgel 60 F_{254} and testosterone and DHT were chromatographed using ethyl acetate/cyclohexane (1:1) as the developing solvent. The plate was air dried, sprayed with primuline solution (10 mg/400 ml in acetone/water (4:1)), and testosterone and DHT were located under the UV lights. Androgen containing areas were cut and the strips were soaked in the 5 ml of Aquasol-2 and radioactivities were counted in the scintillation counter.

pH dependent 5α-reductase activities

To measure pH dependent 5α-reductase activities, the assays were carried out in the reaction mixture containing in a final volume of 200 μl: 1 mM DTT, 40 mM potassium phosphate buffers ranging from pH 4–9, 2 mM NADPH, 2–108 nM [1,2,6,7-^{3}H]testosterone, and the prostatic enzyme fraction. Approximately 50 μg protein of human prostatic enzyme fraction was used for each assay. The reaction was carried out in duplicate at 37°C for 30 min and the radioactivities were counted as above.

Inhibitory effects of drugs

To calculate IC_{50} of the drug, the assays were carried out in the reaction mixture containing in a final volume of 200 μl: 1 mM DTT, 40 mM sodium phosphate buffer pH 6.5, 0.1 mM NADPH, 2 nM [1,2,6,7-^{3}H]-testosterone, and the prostatic enzyme fraction. Amounts of human (10–15 μg protein), rat (10–15 μg protein), monkey (40–45 μg protein), and dog (approx. 90 μg protein) prostatic enzyme fractions were settled to adjust the rate of conversion of testosterone into

DHT to around 30% in control tubes. Drugs were added in 20 μl of 10% dimethylsulfoxide (DMSO) whereas control tubes received the same volume of 10% DMSO. The reaction was carried out in duplicate at 37°C for 60 min and the radioactivities were counted as above.

To calculate kinetic parameters including inhibitor constants (K_i) of the drugs, the assays were carried out in the reaction mixture containing in a total volume of 200 μl: 1 mM DTT, 40 mM potassium phosphate buffers pH 5 or 6.5, 2 mM NADPH, 8–108 nM [1,2,6,7-^{3}H]testosterone, and 66 μg protein of human prostatic enzyme fraction. The reaction was carried out in duplicate at 37°C for 25 min.

Enzyme assays

Activities of 3α-hydroxysteroid oxidoreductase and 3β-hydroxysteroid oxidoreductase were assayed as previously described [17, 18].

Dog prostate was homogenized in 20 mM Tris–HCl buffer pH 7.5 and 1 mM ethylenediaminetetraacetate disodium salt (EDTA) and the microsomal fraction was collected by the centrifugation. The reaction mixture of 3α-hydroxysteroid oxidoreductase contained 75 mM potassium phosphate buffer pH 7.0, 0.75 mM EDTA, 0.5 mM NADPH, 3 mM [1,2,4,5,6,7-^{3}H]DHT, and the microsomal fraction. Inhibitor was added in 20 μl of DMSO. The assay was carried out at 30°C for 60 min, stopped by mixing with ethyl acetate. Extracted androgens were spotted on the aluminium oxide coated TLC plate and chromatographed using benzene/ethanol (192:8) as the developing solvent. Androgen containing areas were cut under the UV lights and radioactivities were counted in the scintillation counter.

Rat liver 3α-hydroxysteroid oxidoreductase was separated with ammonium sulfate saturation (from 40 to 75%) and dissolved in the buffer (10 mM Tris–HCl buffer, pH 8.6, 1 mM EDTA, and 1 mM DTT). The reaction mixture contained 100 mM potassium phosphate buffer pH 7.0, 2.3 mM NADH, 75 μM androsterone, and the enzyme fraction. The initial rates of the reactions were determined by continuously monitoring the change of absorption at 340 nm.

Rat ovary was homogenized in the buffer (25 mM Tris–HCl buffer, pH 7.4, 250 mM sucrose, and 1 mM EDTA) and the microsomal fraction was collected by the centrifugation. The reaction mixture of 3β-hydroxysteroid oxidoreductase contained 75 mM potassium phosphate buffer pH 7.0, 0.75 mM EDTA, 0.5 mM NADPH, 30 μM [1,2,4,5,6,7-^{3}H]DHT, and the microsomal fraction. The reaction was carried out at 30°C for 60 min. The radioactivities were counted according to the procedures described above.

Calculation

The inhibitory assay data were processed using a Cricket Graph for a Macintosh computer to draw line Lineweaver–Burk plots and calculate kinetic parameters.

Protein determination

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

RESULTS

pH dependent 5α-reductase activities

Human prostatic 5α-reductase from the nuclear membrane fractions of the prostate demonstrated time-dependent conversion of testosterone into DHT in the presence of NADPH. The pH profile of human 5α-reductase activity was observed by using various concentrations of testosterone (Fig. 2). In the presence of 2 nM testosterone, human 5α-reductase showed the maximal conversion of testosterone into DHT at around pH 6.5. With higher concentrations of testosterone pH optima transferred to more acidic range. In the presence of 58 or 108 nM testosterone, human 5α-reductase showed the maximal activity at around pH 5. The actual conversion rates and the amounts of DHT production at pH 6.5 and 5 are shown in the Table 1, and kinetic parameters were also calculated. The conversion rate is larger at pH 6.5 than at pH 5 with 2 nM testosterone, but opposite results were shown with testosterone at concentrations above 18 nM. These results indicate that the maximal velocity of this enzyme is higher at pH 5 than at pH 6.5 although affinity for testosterone is higher at pH 6.5 than at pH 5. Rat prostatic 5α-reductase also showed the maximal activity at around pH 6.5 with 2 nM testosterone (data not shown).

Inhibitory effects of FK143 on prostatic 5α-reductases

The intraprostatic concentration of testosterone is reported to be lower than 10 nM in various animal

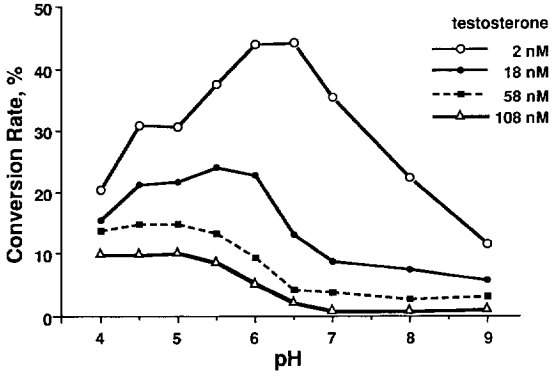

Fig. 2. Effect of testosterone concentration on pH optima of human prostatic 5α-reductase activity. The reaction mixtures contained 2, 18, 58 or 108 nM [^{3}H]testosterone and buffers ranging from pH 4 to 9 and were incubated in duplicate at 37°C for 30 min.

Table 1. Effect of pH on the human prostatic 5α-reductase activity

pH	Testosterone concentration (nM) 2	18	58	108	K_m (nM)	V_{max} (fmol/min)
5						
Conversion rate (%)	30.6	21.7	14.8	10.2	45.8	97.6
DHT production (fmol/min)	4.1	26.0	57.4	73.7		
6.5						
Conversion rate (%)	44.2	13.0	4.3	2.3	4.1	18.1
DHT production (fmol/min)	5.9	15.6	16.7	16.7		

species including human. Moreover, human prostatic 5α-reductase showed high affinity for testosterone at pH 6.5 (Table 1). In consideration of these characteristics, the IC_{50} values of 5α-reductase inhibitors were measured using 2 nM testosterone at pH 6.5 in order to mimic the physiological conditions. Human prostatic 5α-reductase activity was measured in the presence of increasing concentration of FK143 (Fig. 3). FK143 inhibited 5α-reductase activity in a dose-dependent manner and 10^{-7} M FK143 showed complete inhibition.

Prostatic fractions from dog and monkey as well as human and rat prostatic enzymes showed considerable 5α-reductase activities. The conversion rates were adjusted to around 30% using human (10–15 μg protein), rat (10–15 μg protein), monkey (40–45 μg protein), and dog (approx. 90 μg protein) prostatic enzyme fractions in the condition with 2 nM testosterone at pH 6.5. Table 2 comparatively shows the IC_{50} values of FK143 to the 5α-reductases from various animal species. FK143 inhibited human prostatic 5α-reductase with IC_{50} value of 1.9 nM, and it also showed similar inhibitory potencies to 5α-reductases from rat, dog and monkey prostates. Inhibitory potencies of finasteride were also evaluated and almost same IC_{50} values as those of FK143 were obtained in all animal species examined.

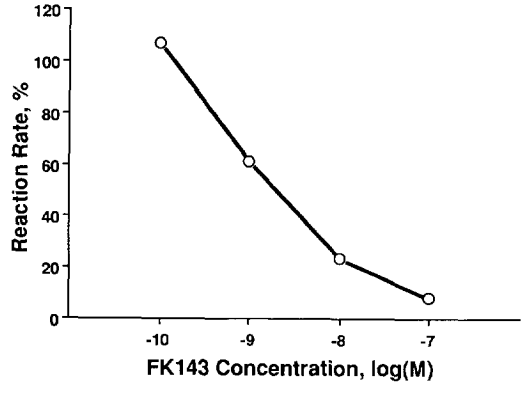

Fig. 3. Inhibitory effect of FK143 on human prostatic 5α-reductase. The reaction mixtures contained 2 nM [^{3}H]testosterone and incubated at 37°C for 60 min at pH 6.5. Results are the average of 3 experiments.

Evaluation of kinetic parameters on human prostatic 5α-reductase

Inhibitory action of FK143 on human 5α-reductase was examined in the presence of several concentrations of drug by using various concentrations of testosterone at pH 6.5. Lineweaver–Burk plots were made according to these data and the kinetic constants and the mode of inhibition were determined. Human prostatic 5α-reductase showed a high affinity for testosterone with a K_m value of 12.1 nM (Table 3). Figure 4(A) indicates a noncompetitive inhibition of human 5α-reductase by FK143. This inhibitory manner is apparently different from the competitive inhibition by finasteride [Fig. 4(B)]. FK143 also inhibited rat prostatic 5α-reductase in a noncompetitive fashion (data not shown). K_i values of FK143 and finasteride on human prostatic 5α-reductase at pH 6.5 are calculated by replotting the results of Lineweaver–Burk analyses (Table 3). Inhibitor constants of FK143 on inhibitor–enzyme complex (Kie) and inhibitor–enzyme–substrate complex (Kies) are 18.1 and 9.6 nM, respectively. Kinetic analyses of FK143 and finasteride were also performed at pH 5 and almost same K_i values as those at pH 6.5 were obtained in both drugs (Table 3).

Effect of testosterone concentration on the inhibitory effect of drugs

Table 4 shows the effect of testosterone concentration on the inhibitory effects of FK143 and finasteride on human prostatic 5α-reductase. Inhibitory ratio of finasteride is high at low concentration of testosterone (18 nM), although the ratios decreased in the higher concentrations of testosterone. On the contrary, inhibitory ratios of various concentrations of FK143 are almost constant regardless of the testosterone concentration.

Specificity of 5α-reductase inhibition by FK143

The inhibitory effects of FK143 on the other androgen related enzymes were estimated (Table 5). The activities of 3α-hydroxysteroid oxidoreductase from dog prostate, 3α-hydroxysteroid oxidoreductase from rat liver and 3β-hydroxysteroid oxidoreductase from rat ovary were assayed by using DHT, andros-

Table 2. IC_{50} of drugs on prostatic 5α-reductases

	IC_{50} (nM)			
	Rat	Dog	Monkey	Human
FK143	4.2 ± 1.0 (3)	4.9 ± 1.5 (4)	4.5 ± 0.5 (3)	1.9 ± 0.9 (3)
Finasteride	5.9 ± 2.1 (3)	5.0 ± 1.7 (3)	5.1 ± 1.0 (3)	1.0 ± 0.1 (4)

Values represent mean ± SE (n).

terone and DHT as the substrate, respectively and IC_{50} values for these enzymes were 20 μM, more than 50 μM, and 12 μM, respectively.

DISCUSSION

The prostate is one of the major 5α-reductase containing organs [1, 19], and 5α-reductase plays the essential role in prostate growth [9, 10]. In this report we examined the prostatic 5α-reductase activities of human and other animal species, and all the prostates showed considerable 5α-reductase activities at around neutral pH.

Recently the existence of two isozymes of human and rat 5α-reductases have been reported [20–24]. Human type 1 and type 2 isozymes showed quite distinct pH profiles and K_m values for testosterone. Type 1 isozyme is active in the broad range around the neutral pH and has a large K_m for testosterone, while type 2 isozyme showed a sharp optimum at around pH 5 and has a small K_m for testosterone [22].

In this study we observed the pH dependent activity of human prostatic 5α-reductase using various concentrations of testosterone, and showed that pH profiles varied according to the testosterone concentrations (Fig. 2). Human prostatic 5α-reductase showed the maximal activity at around pH 5 with 108 nM testosterone. This pH profile is almost the same as those of human type 2 isozyme measured in the presence of more than 0.3 μM testosterone [16, 22, 25]. These results suggest that human prostatic 5α-reductase may be the type 2 isozyme.

On the other hand, human prostatic 5α-reductase showed the maximal activity at around neutral pH with 2 nM testosterone (Fig. 2). Comparing the kinetic parameters at pH 5 and those at pH 6.5, it is indicated that the maximal velocity of this enzyme is higher at pH 5 than at pH 6.5 although affinity for testosterone is higher at pH 6.5 than at pH 5. The reason for this discrepancy is unclear. Thigpen *et al.* speculated that the enzyme had a neutral pH optimum within the cell, although pH optimum might shift to the acidic range because of the disruption of the lipid bilayer of membrane caused by the mechanical destruction of cells [26].

We assayed the 5α-reductase activities of prostatic tissues from 20 patients with BPH separately at pH 6.5 and at pH 7.5 with 2 nM testosterone, and all the specimens showed considerable activities at pH 6.5 while little showed activities at pH 7.5 (data not shown). This result suggests that major component of 5α-reductase activity of the prostates from BPH patients may be attributed to the type 2 isozyme. Recently the mRNA of both isozymes have been located in various human organs, and mRNA of type 2 isozyme is the major component in the prostates from BPH patients [19, 27]. This made a good agreement with our observation. This is also supported by the fact that finasteride, a type 2 selective 5α-reductase inhibitor, showed a high potency to inhibit the human prostatic 5α-reductase (Table 2). Rat prostatic 5α-reductase also showed maximal activity at around neutral pH. Although rat prostate is reported to have both type 1 and type 2 isozymes whose pH profiles are almost identical to human isozymes, it is difficult to distinguish which isozyme mainly contributes to the maximal activity at pH 6.5, because finasteride showed high inhibitory potency to both rat type 1 and type 2 isozymes [24]. It is probable that 5α-reductase activity of rat prostate is a considerable mixture of both isozymes [24, 28].

FK143 is a newly synthesized nonsteroidal inhibitor of 5α-reductase. FK143 showed a high potency to inhibit human prostatic 5α-reductase, and also

Table 3. Kinetic constants of drugs on human prostatic 5α-reductase

		pH 5	pH 6.5
K_m for testosterone		55.9 ± 7.6 (8)	12.1 ± 2.5 (8)
FK143	Kie	19.4 ± 4.0 (3)	18.1 ± 4.5 (4)
	Kies	15.1 ± 0.6 (3)	9.6 ± 3.0 (4)
Finasteride	Kie	0.4 (2)	1.6 (2)

All values are nM concentrations and represent mean ± SE (n ±).

Table 4. Effect of testosterone concentration on the inhibitory effect of drugs on human prostatic 5α-reductase

		Testosterone concentration (nM)		
	n	18	58	108
FK143				
5 nM	3	30.0 ± 4.6	36.2 ± 2.0	36.8 ± 5.3
15 nM	3	57.6 ± 4.2	68.4 ± 4.4	65.8 ± 3.2
30 nM	3	76.7 ± 2.1	79.5 ± 0.6	82.0 ± 1.7
Finasteride				
10 nM	2	73.4	61.3	38.1

Values represent mean ± SE of inhibitory ratio (%). Reactions were carried out at pH 6.5.

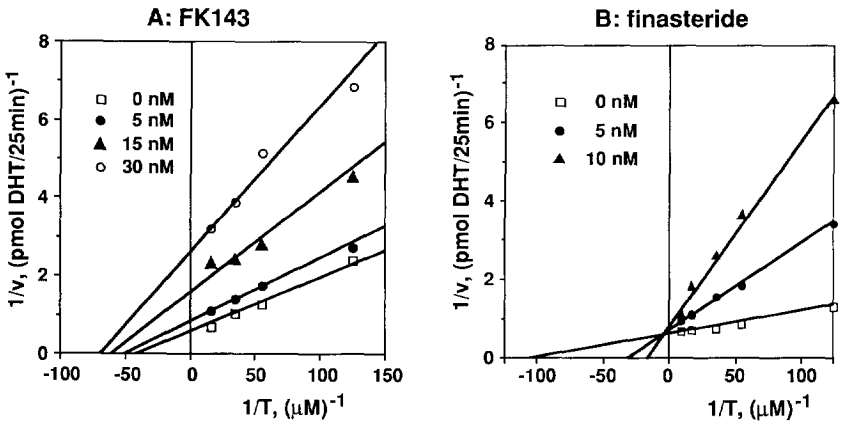

Fig. 4. Lineweaver–Burk plots of the inhibition of human prostatic 5α-reductase by drugs. The reaction mixtures contained various concentrations (8–108 nM) of testosterone and were incubated in the presence of FK143 (A) or finasteride (B) in duplicate at 37°C for 25 min at pH 6.5.

inhibited rat, dog and monkey prostatic 5α-reductase with similar IC_{50} values (Table 2).

K_i values of FK143 on human 5α-reductase at pH 6.5 were larger than that of finasteride, compared with close similarity of IC_{50} values. It is not the difference of testosterone concentration but the difference of protein contents which had possibly increased the K_i value of FK143, as the inhibitory effect of FK143 is not affected by testosterone concentration (Table 4). Ten to fifteen μg protein of enzyme fraction was used in the IC_{50} experiments while 66 μg protein was used in the K_i experiments in order to obtain enough conversion rate. The larger volume of nonenzymatic protein may have attenuated the inhibitory effect of FK143. K_i values of both FK143 and inasteride for human 5α-reductase are similar at pH 6.5 and pH 5, indicating that affinities of both drugs for the enzyme are constant regardless of the pH environment.

Most steroidal compounds including finasteride inhibited 5α-reductase in a competitive fashion except epristeride (SKF-105657), which demonstrated an uncompetitive inhibition [29]. FK143 is a noncompetitive inhibitor of human 5α-reductase (Fig. 4). We have not yet specified the binding site of FK143 on 5α-reductase protein because the enzyme source used in this study is a rather crude one. If the substrate concentration becomes higher, the inhibitory potency becomes weaker in the case of the competitive inhibition because of the competition of the substrate and inhibitor on the active site of the enzyme. However, noncompetitive inhibition may be not affected by the substrate concentration. Although similar IC_{50} values of FK143 and finasteride were obtained with a rather small concentration of testosterone (2 nM, Table 2), we confirmed that higher concentration of testosterone reduced the inhibitory ratio of finasteride to human prostatic 5α-reductase while inhibitory ratio of FK143 are almost constant regardless of the testosterone concentration (Table 4). The evaluation of the therapeutic efficacy of 5α-reductase inhibitors for BPH is now under study [15]. It is well known that accumulation of testosterone occurred in the prostates of animals or patients who received 5α-reductase inhibitors [12, 13, 30, 31]. Accumulated testosterone will compete with the competitive 5α-reductase inhibitors on the active site of the enzyme and will attenuate the inhibitory efficacy. This is supposed to be one of the factors of the incomplete involution of the prostate glands by the competitive 5α-reductase inhibitors. Noncompetitive inhibition of 5α-reductase may be not affected by the accumulation of testosterone and be able to fully inhibit the enzyme activity. It is expected that noncompetitive 5α-reductase inhibitor may provide a new approach for the BPH treatment.

In this report we studied *in vitro* effects of a newly synthesized 5α-reductase inhibitor, FK143. FK143 has a nonsteroidal structure and showed a noncompetitive inhibition of 5α-reductase while it showed no inhibitory effects on the other androgen related oxidoreductases examined. Further studies are required to evaluate the inhibitory action of FK143 on each isozyme.

Acknowledgements—We thank Dr M. Tachibana at Hospital of Keio University for providing us human prostatic tissues.

Table 5. Inhibitory effects of FK143 on the androgen-related enzymes

Enzyme	Origin	Substrate	IC_{50} (M)
3α-Hydroxysteroid oxidoreductase	Dog prostate	DHT	2.0×10^{-5}
3α-Hydroxysteroid oxidoreductase	Rat liver	Androsterone	$>5.0 \times 10^{-5}$
3β-Hydroxysteroid oxidoreductase	Rat ovary	DHT	1.2×10^{-5}

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