

## Hormonal Effects of Z-350, Possessing Steroid 5 $\alpha$ -Reductase Inhibitory and $\alpha_1$ -Adrenoceptor Antagonistic Actions, in the Rat

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**ABSTRACT**—We examined the hormonal effects of Z-350, (*S*)-4-[3-(4-{1-(4-methylphenyl)-3-[4-(2-methoxyphenyl)piperazine-1-yl]propoxy}benzoyl)indole-1-yl]butyric acid hydrochloride, which has both  $\alpha_1$ -adrenoceptor blocking activity and steroid 5 $\alpha$ -reductase inhibitory activity, in male and female rats. Z-350 administered orally for 14 days at a dose of 30 mg/kg to normal male rats significantly reduced the weight of the prostate and seminal vesicles without affecting the weight of the testis, epididymis, adrenals, kidney or liver. Prostatic levels of dihydrotestosterone decreased dose-dependently, with a slight increase in the level of testosterone at a Z-350 dose of 100 mg/kg. We observed no effects on the weight of the prostate in castrated rats or on the weight of the uterus in normal or 17 $\beta$ -estradiol-treated female rats. These results suggest that Z-350 inhibits prostatic growth via inhibition of steroid 5-reductase without other hormonal effects.

**Keywords:** Z-350, Steroid 5 $\alpha$ -reductase, Benign prostatic hyperplasia, Testosterone, Dihydrotestosterone

Benign prostatic hyperplasia (BPH), which is a common disorder in aging men, is associated with growth of prostatic glandular and stromal elements. BPH causes symptoms in the lower urinary tract and reduces quality of life.

The lower urinary tract symptoms caused by BPH are attributed to two main components: a dynamic component involving excessive contraction of prostatic smooth muscle mediated by  $\alpha_1$ -adrenoceptors (1–4) and a static component involving obstruction of the urethra by the enlarged prostate (5).  $\alpha_1$ -Adrenoceptor blockers (6–10) represent a palliative approach to therapy for lower urinary tract symptoms caused by BPH and are effective in alleviating dynamic obstruction. On the other hand, anti-androgens can improve static obstruction by reducing prostatic mass, although they have adverse effects on sexual function, including impotence and decreased libido, which is caused by plasma testosterone deprivation (5).

Steroid 5 $\alpha$ -reductase (EC 1.3.1.22; 5 $\alpha$ -R), a rate-limiting enzyme that converts testosterone to the potent androgen dihydrotestosterone, plays a critical role in prostatic growth (11). Finasteride is a specific 5 $\alpha$ -R inhibitor that can reduce prostate volume with fewer adverse effects on sexual function than anti-androgens (12–15), and it has already been approved for clinical use in the US and Europe.

On the basis of the concept that combined activity (i.e.,  $\alpha_1$ -adrenoceptor blocking and 5 $\alpha$ -R inhibition) may be more beneficial for the treatment of BPH than each activity alone, we recently reported the pharmacological profile of Z-350, which possesses  $\alpha_1$ -adrenoceptor blocking and 5 $\alpha$ -R inhibitory activity, *in vitro* (16) and *in vivo* (17). Z-350 inhibited rat prostatic 5 $\alpha$ -R in a non-competitive manner and prevented testosterone-induced re-growth of the prostate in castrated rats.

In the present study, we investigated the effects of Z-350 on the weight of the testis, epididymis, adrenal gland and other organs in normal male rats to evaluate the hormonal effects. We also investigated the effects of Z-350 on the prostate in castrated male rats and on the uterus in normal and 17 $\beta$ -estradiol-treated female rats to determine its androgenic, estrogenic and anti-estrogenic actions.

### MATERIALS AND METHODS

#### *Experimental animals*

Male and female Sprague-Dawley rats were obtained from Charles River Japan (Shizuoka). Animals were housed in a controlled environment at 23  $\pm$  3°C with a 12-h light and dark cycle and were acclimated for 1 week before use. All animals were allowed free access to food (CRF-1; Oriental Yeast, Tokyo) and tap water unless otherwise stated.

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### Effects in normal male rats

Z-350 and finasteride were administered orally once a day for 14 days at doses of 3–30 mg/kg and 1–10 mg/kg, respectively, to 7-week-old male rats. After the last dose, rats were fasted for 18 h and then sacrificed by cervical dislocation under anesthesia with ethyl ether. The prostate, seminal vesicles and other organs (epididymes, testes, adrenals, liver and kidneys) were removed and weighed.

### Prostatic levels of testosterone and dihydrotestosterone in normal male rats

Z-350 (10 or 100 mg/kg) was administered and the ventral prostate was removed as above. Prostatic concentrations of testosterone and dihydrotestosterone were measured according to previously described methods (18), with slight modifications. The prostate was homogenized with a Teflon-glass homogenizer in 3 ml water and incubated at 70°C for 2 min. The homogenate was extracted with 3 ml hexane and evaporated under a nitrogen stream. The residue was treated with 45  $\mu$ l *N,O*-bis(trimethylsilyl)acetamide / trimethylchlorosilane / pyridine (1:4:4) at room temperature for 1 h and dissolved in 45  $\mu$ l hexane containing 0.0001% hexachlorbenzene and then injected in GC-MS (column: DB-1, 30 m  $\times$  0.32 mm  $\times$  0.1  $\mu$ m; J & W, Folsom, CA, USA).

### Effects in castrated male rats

Four-week-old male rats were castrated under ether anesthesia. Four days after interval, Z-350 (10, 30 and 100 mg/kg) was administered orally or testosterone propionate (100  $\mu$ g/animal) was injected subcutaneously once a day for 4 days. The ventral prostate and seminal vesicles were removed and weighed as above.

### Effects in normal and 17 $\beta$ -estradiol-treated female rats

Z-350 (10–100 mg/kg) were given orally once a day for 4 days to 4-week-old normal female rats and female rats treated with 17 $\beta$ -estradiol (100  $\mu$ g/animal subcutaneously) (19). The uterus was removed and weighed as above.

### Drugs

Z-350 and finasteride were synthesized at Zeria Pharmaceutical (Saitama). Testosterone propionate (Enarmon injection) was purchased from Teikoku Zoki (Tokyo). 17 $\beta$ -Estradiol was purchased from Sigma (St. Louis, MO, USA). Other reagents were of the highest grade available.

Z-350 and finasteride were suspended in 0.5% (w/v) methylcellulose solution just before administration and administered at 10 ml/kg. Testosterone propionate and 17 $\beta$ -estradiol were dissolved in sesame oil.

### Statistical analyses

Data are expressed as the mean and S.E.M. and were

subjected to analysis of variance (ANOVA). Statistical evaluation was carried out using Dunnett's multiple test; differences at  $P < 0.05$  were considered significant.

## RESULTS

### Effects in normal male rats

Figure 1 shows the effects of Z-350 and finasteride on the weights of the prostate and seminal vesicles in normal male rats. Z-350 significantly reduced the weights of the prostate and seminal vesicles at 30 mg/kg, and finasteride had similar effects at a dose of 1 mg/kg. Z-350 had no effects on the weights of other organs, but finasteride at 10 mg/kg slightly but significantly decreased the weight of the epididymis (Table 1).

### Prostatic levels of testosterone and dihydrotestosterone in normal male rats

The weight of the prostate was significantly reduced by oral administration of Z-350 for 14 days at doses of 10 and 100 mg/kg (Table 2). Prostatic levels of dihydro-

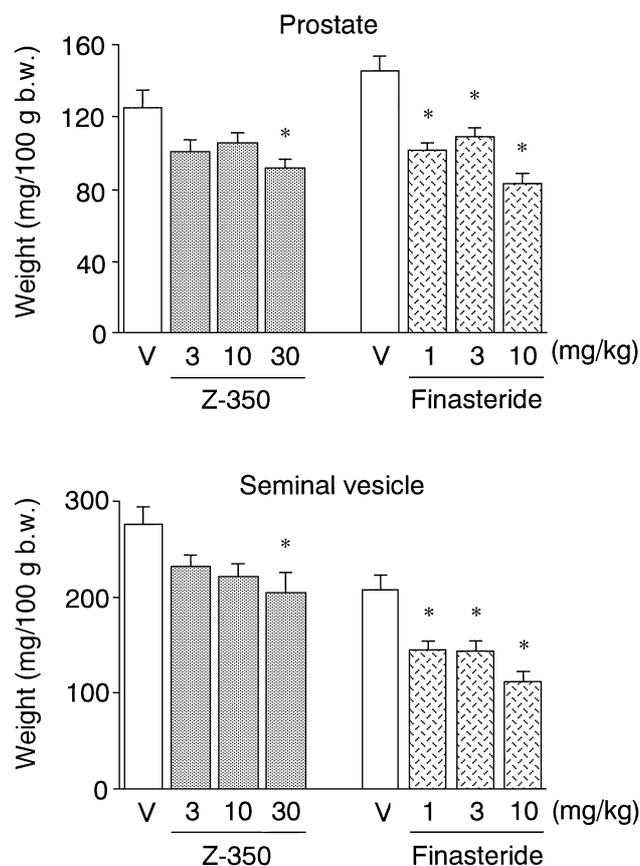


Fig. 1. Effects of Z-350 and finasteride on the weight of the prostate and seminal vesicles in normal rats. Drugs were administered for 14 days. Each column represents the mean  $\pm$  S.E.M. of 8 rats. V, vehicle. \* $P < 0.05$  vs vehicle (Dunnett's two-tailed test).

**Table 1.** Effects of Z-350 and finasteride on the weights of epididymis, testis, adrenal, liver and kidney in normal male rats

	mg/kg (p.o.)	n	Organ weight				
			Epididymis*	Tastis**	Adrenal*	Liver**	Kidney**
Vehicle		8	223.0 ± 14.0	0.958 ± 0.02	17.12 ± 0.80	3.413 ± 0.17	0.836 ± 0.02
Z-350	3	7	208.0 ± 10.9	0.924 ± 0.02	16.77 ± 0.59	3.520 ± 0.21	0.844 ± 0.02
	10	8	226.8 ± 7.5	0.986 ± 0.03	18.61 ± 0.66	3.176 ± 0.09	0.840 ± 0.03
	30	8	197.4 ± 3.9	0.935 ± 0.02	15.92 ± 0.47	3.186 ± 0.06	0.836 ± 0.03
Vehicle		8	195.2 ± 12.5	0.884 ± 0.04	14.88 ± 0.37	3.378 ± 0.14	0.809 ± 0.04
Finasteride	1	8	175.0 ± 14.7	0.933 ± 0.04	13.27 ± 0.61	3.212 ± 0.06	0.836 ± 0.01
	3	8	181.2 ± 6.1	0.907 ± 0.03	14.86 ± 0.64	3.335 ± 0.11	0.825 ± 0.02
	10	8	158.5 ± 6.7 <sup>#</sup>	0.892 ± 0.04	13.41 ± 0.75	3.199 ± 0.08	0.770 ± 0.02

Z-350 and finasteride were orally administered for 14 days to normal male rats. Each value represents the mean ± S.E.M. of 7 or 8 rats. \*mg/100 g body weight, \*\*g/100 g body weight. <sup>#</sup>*P*<0.05 vs vehicle group.

**Table 2.** Effects of Z-350 on the prostatic weights and the prostatic contents of testosterone and dihydrotestosterone in normal male rats

	mg/kg (p.o.)	Prostatic weight (mg)	Prostatic contents			
			Testosterone		Dihydrotestosterone	
			(ng/prostate)	(ng/g tissue)	(ng/prostate)	(ng/g tissue)
Vehicle		285.0 ± 13.0	0.33 ± 0.04	1.19 ± 0.16	1.33 ± 0.13	4.69 ± 0.40
Z-350	10	212.7 ± 17.7*	0.40 ± 0.05	2.00 ± 0.34	0.81 ± 0.14*	3.85 ± 0.57
	100	176.6 ± 11.3*	0.45 ± 0.09	2.63 ± 0.57*	0.55 ± 0.12*	3.07 ± 0.59

Z-350 was orally administered for 14 days to normal male rats. Each value represents the mean ± S.E.M. of 8 or 9 rats. \**P*<0.05 vs vehicle group.

testosterone decreased dose-dependently, with a slight increase in testosterone content, after administration of Z-350 (Table 2).

#### Effects in castrated male rats

The weights of the prostate and seminal vesicles in castrated rats after 4 days of oral administration of Z-350 or subcutaneous injection of testosterone propionate are shown in Fig. 2. Z-350 (10–100 mg/kg) did not affect the weight of the prostate or seminal vesicles; conversely, testosterone propionate (100 µg/animal) dramatically increased the weight of the prostate and seminal vesicles in castrated rats.

#### Effect in normal and 17β-estradiol-treated female rats

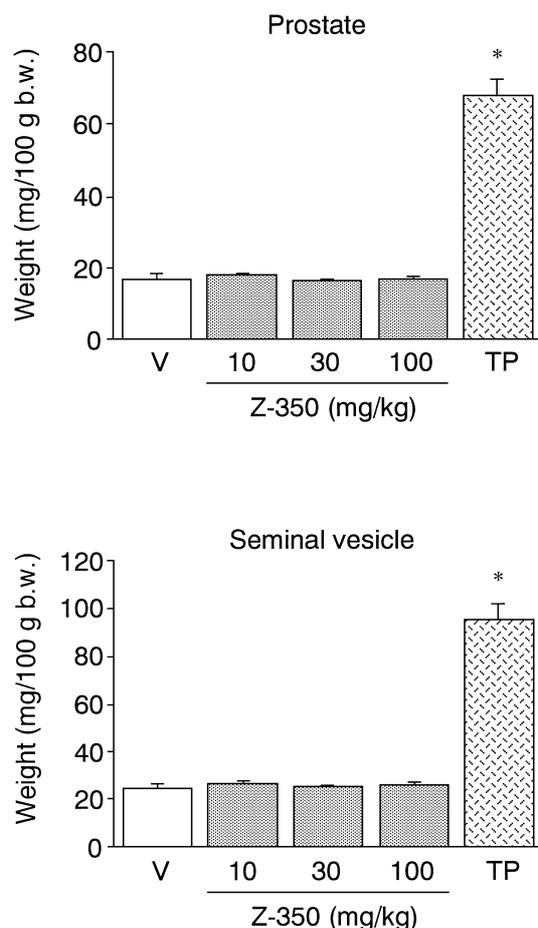
Z-350 had no effect on the weight of the uterus in normal and 17β-estradiol-treated female rats (Fig. 3).

## DISCUSSION

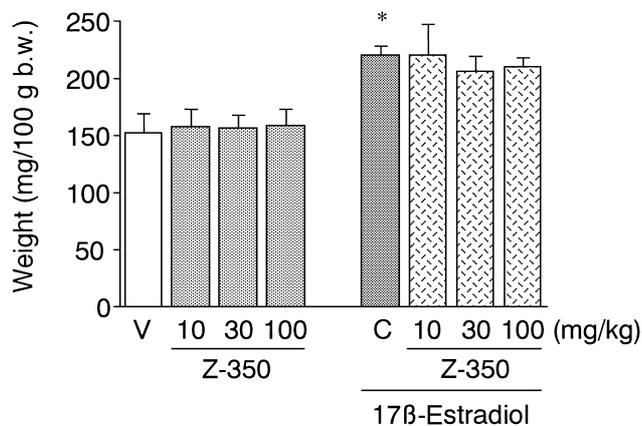
We investigated the effects of Z-350 on several tissues in normal male rats, on the prostate of castrated male rats and on the uterus of female rats, with the aim of clarifying its hormonal effects other than 5α-R inhibition.

Z-350 reduced the weights of the prostate and seminal

vesicles in normal male rats. It is well known that the prostate is an androgen-, especially dihydrotestosterone-, dependent tissue and that dihydrotestosterone plays a critical role in prostatic growth and function (11). The dose of Z-350 required for a significant reduction of prostatic weight in normal rats was 30 mg/kg. This was higher than that previously reported for the inhibition of testosterone-induced prostatic growth in castrated rats (17). Similar observations have been reported previously. For example, prostatic growth induced by testosterone in castrated rats is inhibited by turosteride at doses of 1–10 mg/kg (20) and by CGP53153 at doses of 0.01–3 mg/kg (21). On the other hand, the prostatic weights of normal rats are reduced by turosteride at doses of 3–30 mg/kg (22) and by CGP53153 at doses of 1–10 mg/kg (21). This indicates that the doses of these compounds required to reduce prostatic weight in normal rats are over three times higher than those required to inhibit prostatic growth induced by testosterone in castrated rats. This difference is thought to be due to the fact that endogenous testosterone in normal rats could partly counteract the effect of Z-350 on the androgen receptor, resulting in prostatic growth (23). In contrast, in castrated rats, exogenous testosterone would mostly be converted to dihydrotestosterone by prostatic 5α-R, indicating that prostatic growth may be dependent on 5α-R



**Fig. 2.** Effects of Z-350 and testosterone propionate (TP) on the weight of the prostate and seminal vesicles in castrated rats. Four days after castration, TP and Z-350 were administered for 4 days. Each column represents the mean  $\pm$  S.E.M. of 8 rats. V, vehicle; TP, testosterone propionate (100  $\mu$ g/animal, s.c.). \* $P$ <0.05 vs vehicle (Dunnett's two-tailed test).



**Fig. 3.** Effects of Z-350 on the weight of the uterus in normal and 17 $\beta$ -estradiol-treated female rats. Z-350 was administered for 4 days to normal and  $\beta$ -estradiol-treated (100  $\mu$ g/animal, s.c.) female rats. Each column represents the mean  $\pm$  S.E.M. of 6 rats. V, vehicle. \* $P$ <0.05 vs vehicle (Dunnett's two-tailed test).

activity. In fact, the prostatic 5 $\alpha$ -R activity of castrated rats is about three times higher than that of normal rats (24).

Prostatic 5 $\alpha$ -R inhibition by Z-350 was confirmed by determination of prostatic levels of testosterone and dihydrotestosterone after oral administration of Z-350 in normal rats for 14 days. Z-350 reduced prostatic content of dihydrotestosterone but slightly increased that of testosterone. It has previously been reported that the reduction in prostatic dihydrotestosterone levels caused by finasteride, a competitive inhibitor of 5 $\alpha$ -R (25), is accompanied by an apparent increase in prostatic testosterone levels (26), and it has been suggested that the inhibitory effect of finasteride may gradually diminish because of these increased testosterone levels (22). The increased testosterone level would not be expected to interfere with the inhibitory action of Z-350, since Z-350 inhibits prostatic 5 $\alpha$ -R non-competitively (16). Thus, the inhibitory potency of Z-350 would probably not be influenced by increased prostatic testosterone levels.

The steroidal anti-androgens, e.g., allylestrenol and chlormadinone acetate, affect the weights of the testis, epididymis and adrenal gland (27–29) through plasma testosterone deprivation via the negative feedback inhibition of the hypothalamus (30). Oral administration of Z-350 did not affect the weights of these organs, suggesting that Z-350 does not affect plasma testosterone concentrations. Furthermore, Z-350 showed no effects on either the weight of the prostate and seminal vesicles in castrated rats or the weight of the uterus in normal or 17 $\beta$ -estradiol-treated female rats, indicating that Z-350 has no androgenic, estrogenic or anti-estrogenic activity. These findings, together with our recent report that Z-350 does not reduce prostatic growth induced by dihydrotestosterone in castrated rats (17), indicate that Z-350 reduces prostatic weight only by inhibition of 5 $\alpha$ -R, without affecting serum hormonal levels. This result is in agreement with those for other 5 $\alpha$ -R inhibitors (19, 31).

The effects of Z-350 on plasma hormonal levels are not yet clear, so it is very important to evaluate the effects of administration of this drug for long periods on the levels of hormones such as androgens, estrogens, prolactin and luteinizing hormone. Further investigations are required to determine whether Z-350 has an adequate margin of safety for human use.

Recently, it has been demonstrated that the  $\alpha_1$ -antagonists, terazosin and doxazosin, induce prostate apoptosis (32, 33). This apoptotic effect of the  $\alpha_1$ -blockers was not attenuated by simultaneous addition of agonists (33). Moreover, another class of  $\alpha_1$ -blocker, the sulphonamide derivative tamsulosin, had no effects (33). Taken together, these results suggest that the effects of terazosin and doxazosin may be dependent on the quinazoline structure. The structure of Z-350 differs from that of quinazoline. However, it is difficult to investigate the  $\alpha_1$ -blocking activity of Z-350

on prostatic growth, because of its own  $5\alpha$ -R inhibitory effect.

In conclusion, our results indicate that Z-350 should reduce prostatic mass by inhibiting only  $5\alpha$ -R, without other hormonal effects. We expect that Z-350, which concomitantly blocks  $\alpha_1$ -adrenoceptors and inhibits  $5\alpha$ -R, may be a new candidate compound for the treatment of BPH, with additional benefits and limited hormonal adverse effects.

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