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European Journal of Pharmacology 426 (2001) 105–111

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# Pharmacodynamic analysis of steroid 5α-reductase inhibitory actions of Z-350 in rat prostate

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Received 29 May 2001; received in revised form 6 July 2001; accepted 10 July 2001

**Abstract**

The pharmacodynamics of (*S*)-4-{3-{4-{1-(4-methylphenyl)-3-[4-(2-methoxyphenyl)piperazine-1-yl]propoxy}benzoyl}indole-1-yl} butyric acid hydrochloride (Z-350), which has $\alpha_1$-adrenoceptor antagonistic and steroid 5α-reductase inhibitory effects, were investigated in rats. The disposition of Z-350 was a function of linear kinetics at doses from 1 to 30 mg/kg; the bioavailability was calculated to be 65.2%. The inhibition of 5α-reductase was dependent on the concentration of Z-350 in plasma and in the prostate. Analysis of the relationship between the concentration in the prostate and the inhibition seen after a single oral administration showed that the Hill constant was almost 1.0 and $EC_{50}^{n_H}$ was 47.4 ng/g of tissue; these parameters did not change after multiple administration. Z-350 inhibited 5α-reductase 1 h after oral administration at a dose of 3 mg/kg; maximum inhibition was observed after 2–4 h, and the inhibition (%) was maintained for 24 h after oral administration. 

*Keywords:* Z-350; 5α-Reductase; Phamacodynamics; Plasma concentration; Prostate concentration

## 1. Introduction

Benign prostatic hypertrophy is a progressive enlargement of the prostate, which occurs with increasing frequency as men age (Yamada et al., 1994). $\alpha_1$-Adrenoceptors (which are localized in the human prostate) have been implicated in the regulation of bladder outlet resistance. Clinical studies have confirmed that $\alpha_1$-adrenoceptor antagonists (such as prazosin or tamsulosin) significantly increase the urinary flow rate in men with symptomatic benign prostatic hypertrophy (Yamada et al., 1994).

The growth of the prostate gland is dependent on the tissue androgen content, so antiandrogens have been used to treat benign prostatic hypertrophy (Craig et al., 1987). However, a major disadvantage of antiandrogens is their severe side effects, such as sexual dysfunction caused by depletion of testosterone. Finasteride (a steroid 5α-reductase inhibitor) is available for clinical use to reduce the side effects of antiandrogens (Rittmaster et al., 1989; Gormley et al., 1990). (*S*)-4-{3-{4-{1-(4-methylphenyl)-3-[4-(2-methoxyphenyl) piperazine-1-yl] propoxy}benzoyl}indole-1-yl} butyric acid hydrochloride (Z-350) (Fig. 1) is a new compound with $\alpha_1$- adrenoceptor antagonistic and steroid 5α-reductase inhibitory effects. It was developed by Zeria Pharmaceutical (Tokyo, Japan) based on the fact that the mechanical and dynamic components of bladder outlet obstruction (which is associated with benign prostatic hypertrophy) are closely related (Sato et al., 1997; Fukuda et al., 1998, 1999, 2001; Fukuta et al., 1998, 1999). Z-350 inhibited the phenylephrine-induced increase in urethral pressure by acting on $\alpha_1$-adrenoceptors in rabbits, while having effect on mean blood pressure. The inhibitory effect of Z-350 on steroid 5α-reductase was still detectable 8 h after oral administration of Z-350 to rats (Fukuda et al., 1999). Furthermore, Z-350 prevented the prostatic growth that is usually induced by testosterone in castrated rats (Fukuta et al., 1999; Fukuda et al., 2001). Accordingly, analysis of the concentration-time profile of Z-350 in the plasma and prostate could provide meaningful information about the duration of the pharmacological

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PII: S0014-2999(01)01208-0

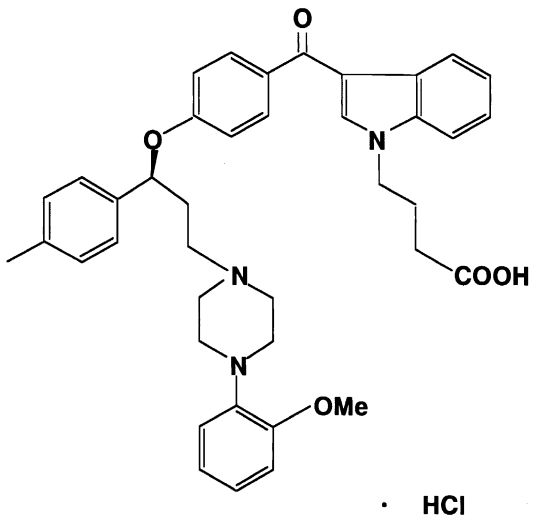


Fig. 1. Chemical structure of Z-350.

action. In this study, we analyzed the relationship between the inhibition of 5α-reductase and the concentrations of Z-350 in plasma and prostate using a pharmacodynamic model. In addition, the effects of dose on the duration of the pharmacological actions were analyzed.

## 2. Materials and methods

### 2.1. Drugs

Z-350 and an internal standard, [(*S*)-4-{3-{4-{1-(4-methylphenyl)-3-[4-(2-methoxyphenyl)piperazine-1-yl]propoxy}-3-methoxybenzoyl}indole-1-yl}butyric acid potassium salt], were synthesized at Zeria Pharmaceutical.

### 2.2. Animals

Male Sprague–Dawley rats (7 or 8 weeks old and weighing approximately 200 g) were purchased from Charles River Japan (Tsukuba, Japan) and were acclimatized to our facilities for 1 week before use. The animals were housed in an animal room maintained at $23 \pm 2$ °C and $55 \pm 10\%$ relative humidity.

### 2.3. Method of administration and tissue preparation

Z-350 was suspended in a 0.5% (w/v) methyl cellulose solution using an agate mortar and then administered orally at doses of 1, 3, 10 and 30 mg/5 ml/kg. For intravenous administration, Z-350 (potassium salt) was dissolved in a saline solution of 10% ethanol at a dose of 1 mg/ml/kg. The rats were fasted for 16 h prior to dosing but were allowed free access to water. The drugs were administered orally or intravenously via the femoral vein. The rats were killed under ether anesthesia by withdrawing blood from the abdominal aorta with a heparinized syringe at scheduled time intervals. Plasma samples were obtained by centrifugation of whole blood at $1000 \times g$ for 10 min. The isolated prostate glands were weighed and homogenized with a Polytron homogenizer in two tissue volumes of ice-cold buffer A (50 mM Tris–HCl buffer, pH7.2, 0.25 M sucrose, 1 mM dithiothreitol). The plasma and homogenates of the prostate were stored at −80 °C before assay.

### 2.4. Assay of Z-350 in plasma and prostate

Five hundred-microliter aliquots of plasma were mixed with 0.7 ml of buffer A, or 200-μl aliquots of prostate homogenate were mixed with 1 ml of buffer A. From each mixed solution, after the addition of 10 μl of internal standard solution (500 μg/ml) and 10 μl of methanol (no additive or containing Z-350 standard), Z-350 was extracted into 5 ml of diethylether, with shaking for 10 min. After centrifugation ($1000 \times g$, 10 min), the organic layer was removed and dried under nitrogen. The residue was dissolved in 0.2 ml of a mobile phase and 70 μl of this solution was injected into the HPLC system.

A Shimadzu LC-9A high-performance liquid chromatography system (Kyoto, Japan) with a system controller (SCL-6B, Shimadzu) and an auto injector (SIL-6B, Shimadzu) was used for analysis. A UV detector set at 326 nm (SPD-6A, Shimadzu) was used to monitor the effluent and to be detect Z-350. The mobile phase, 5 mM phosphate buffer (pH 7.4)–acetonitrile (60:40), was pumped through an analytical column (Capcell Pak C18, SG-120, $250 \times 4.6$ mm (I.D.), Shiseido, Tokyo, Japan) equipped with a pre-column (Lichrospher RP-18, Merck, Darmstadt, Germany) at a flow rate of 1 ml/min. The column was kept at 40 °C using a column oven (CTO-6A, Shimadzu) and recordings were made with a chromatopack (C-R7A, Shimadzu).

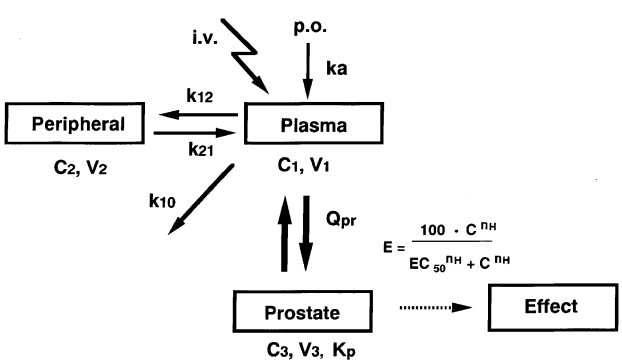


Fig. 2. Pharmacodynamic model of Z-350 after intravenous or oral administration of Z-350 to male rats.

Table 1
Concentrations of Z-350 in plasma and prostate and 5α-reductase inhibition at 2 h after a single oral administration to rats

| Dose (mg/kg) | Concentration | | 5α-Reductase inhibition (%) |
|---|---|---|---|
| | Plasma (ng/ml) | Prostate (ng/g of tissue) | |
| 1 | 91.0 ± 7.2 | 27.4 ± 4.8 | 9.3 ± 19.2 |
| 3 | 473.0 ± 57.6 | 120.2 ± 13.9 | 60.9 ± 3.8 |
| 10 | 2333.3 ± 492.3 | 652.0 ± 87.9 | 90.1 ± 0.9 |
| 30 | 7112.8 ± 909.6 | 1784.3 ± 26.4 | N.T. |

Data are the means ± S.E. from three to five animals.
N.T.: not tested.

### 2.5. Assay of 5α-reductase

Steroid 5α-reductase was measured as described previously (Hirosumi et al., 1995) with minor modifications. The assay of steroid 5α-reductase activity was performed in a final volume of 500 μl containing 50–100 mM Tris–HCl buffer (pH 7.0), 0.5 mM NADPH, 1.4 μM [4-$^{14}$C]testosterone (2.1 GBq/mmol, Du Pont-NEN Research Products, Boston, MA). Five microliters of inhibitors was added to the mixture and the reaction was started by adding an enzyme source (1 mg protein/ml). The reaction mixture was incubated for 60 min at 37 °C, whereafter the reaction was stopped by the addition of 2 ml of ice-cold ethyl acetate and the mixtures were shaken for 5 min and then centrifuged ($1000 \times g$, 5 min). The organic phase was separated and evaporated under nitrogen to dryness, and the residues were dissolved in ethyl acetate and applied to TLC plate (Kieselgel 60 F254, Merck). The plates were developed twice in ethylacetate–cyclohexane (1:1). The radioactive spots were detected by autoradiography, and the areas associated with testosterone and its metabolite, 5α-dihydrotestosterone, were scraped into vials with Aquazol-2 (Du Pont-NEN). Radioactivities were counted with a scintillation counter (Packard, Chicago, IL), and the extent of 5α-reductase inhibition was calculated from the difference in the control and inhibited activities.

### 2.6. Assay of protein

The protein concentration of each homogenate was measured at 562 nm with a spectrophotometer (U-2000, Hitachi, Tokyo), using a BCA reagent (Smith et al., 1985) with bovine serum albumin as standard.

### 2.7. Calculation of pharmacokinetic and pharmacodynamic parameters

Model-independent pharmacokinetic parameters were calculated based on plasma levels of Z-350. These parameters included the time required to reach the maximal concentration ($T_{max}$), the maximal concentration ($C_{max}$), the area under the plasma concentration–time curve (AUC) and the plasma clearance (CL/F). The half-life ($t_{1/2}$) and AUC were calculated by extrapolation from the slope to infinite values during the final phase using the trapezoidal method. The $CL/F$ was calculated by dividing the dose by AUC.

The relationship between the prostate concentration and inhibition of 5α-reductase was described by the Hill equation (Holford and Sheiner, 1981; Hatanaka et al., 1988) (Eq. (1))

$$E(C) = E_{max} C^{n_H} / (EC_{50}^{n_H} + C^{n_H}) \quad (1)$$

where $E(C)$ is the observed effect at the prostate concentration $C$, $n_H$ is the Hill constant, $E_{max}$ is the maximal effect, and $EC_{50}^{n_H}$ is the prostate concentration at half-maximal effect.

The equations were fitted to the data for the prostate concentration and 5α-reductase inhibition after single or multiple oral administration using a non-linear least-squares regression program (MULTI) (Yamaoka et al., 1981) with $E_{max}$ at 100%.

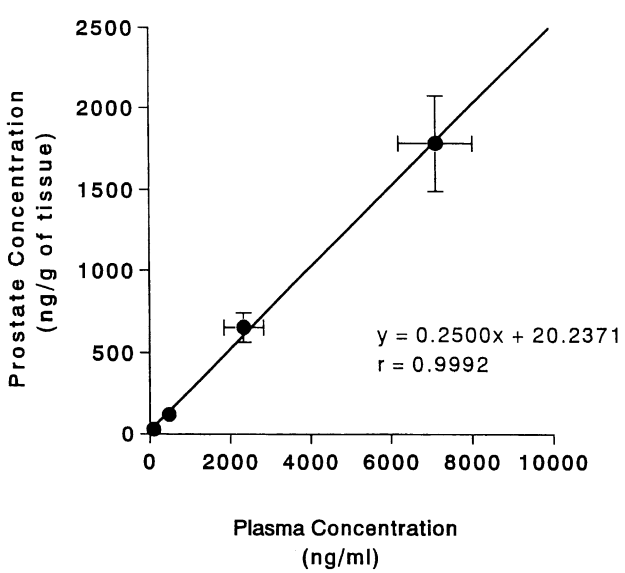


Fig. 3. Relationships between the plasma and prostate concentrations of Z-350 after a single oral administration to rats. Data are the mean ± S.E. values for three or five rats.

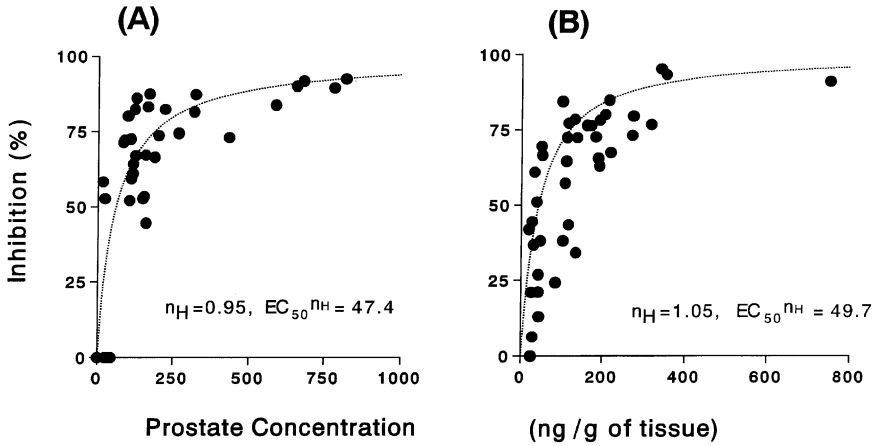


Fig. 4. Relationships between the prostate concentration of Z-350 and the inhibition of 5α-reductase activity after single (A) or multiple (B) oral administration of Z-350 to rats. The dotted line shows the values calculated using the $E_{max}$ model analysis.

The concentrations in plasma and prostate, and 5α-reductase inhibition for all doses were fitted simultaneously to a pharmacodynamic model (Fig. 2). The prostate concentration was calculated according to the conventional blood flow rate-limited model (Inagaki et al., 1988). The pharmacodynamic parameters were calculated by solving

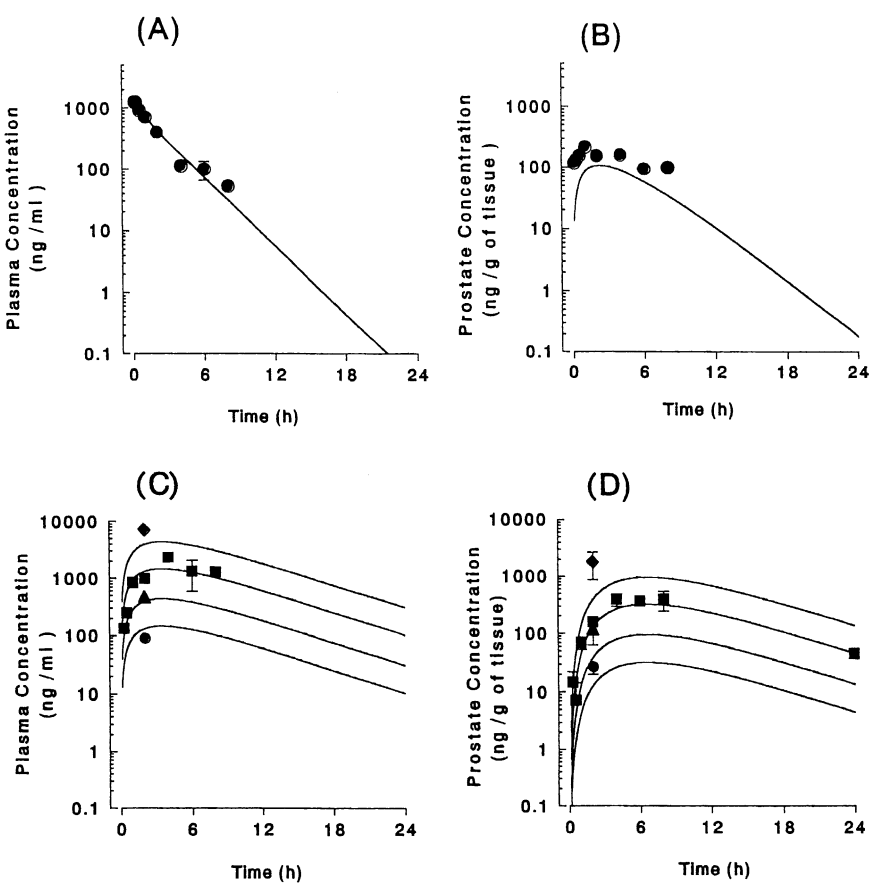


Fig. 5. Plasma and prostate concentrations of Z-350 after intravenous (A, C) and oral (B, D) administration to rats (1 mg/kg: circles, 3 mg/kg: triangles, 10 mg/kg: squares, 30 mg/kg: inverted triangles). Data are the mean ± S.E. values for three or five rats. The solid line shows the calculated values at doses of 1, 3, 10 and 30 mg/kg, calculated using the pharmacodynamic model shown in Fig. 2.

the following differential equations (Eqs. (2)–(6)) and the Hill equation (Eq. (1)), by means of the Runge–Kutta–Gill method (Yamaoka and Nakagawa, 1983).

$$dC_1/dt = k_a FX/V_1 - (k_{12} + k_{10})(C_1 + k_{12})C_2 \quad (2)$$

$$dC_1/dt = -(k_{12} + k_{10})(C_1 + k_{12})C_2 \quad (3)$$

$$dC_2/dt = k_{21}(C_1 - C_2) \quad (4)$$

$$dC_3/dt = Q_{pr}(C_1 - C_3/K_p)/V_3 \quad (5)$$

$$dX/dt = -k_a FX \quad (6)$$

where $C$ is the concentration of Z-350 in each compartment, $k$ is the rate constant between the indicated compartments, $V$ is the distribution volume of each compartment, $K_p$ is the distribution ratio of prostate to plasma, and $Q_{pr}$ is the plasma flow rate in the prostate. $X$ is the amount of a given drug at the site of absorption and $F$ is the bioavailability of Z-350. The plasma concentrations were fitted to Eq. (2) for oral administration and Eq. (3) for intravenous administration.

## 3. Results

### 3.1. Plasma and prostate concentrations, and 5α-reductase inhibition action after a single administration

The plasma and prostate concentrations, and 5α-reductase inhibition at 2 h after a single oral administration of Z-350 to rats at doses of 1, 3, 10 and 30 mg/kg are shown in Table 1. The increases in the concentrations of Z-350 in the plasma and the prostate paralleled the increases in dose. The plots of prostate concentration against plasma concentration were fitted by linear regression ($r = 0.999$) with a slope of 0.25 and a small intercept (Fig. 3). Inhibition of 5α-reductase was enhanced in proportion to dose, and the pharmacological parameters obtained from the Hill equation, $n_H$ and $EC_{50}^{n_H}$, were 0.95 and 47.4 ng/g of tissue, respectively (Fig. 4A).

The time course of Z-350 in plasma and prostate after intravenous (1 mg/kg) and oral (10 mg/kg) administration is shown in Fig. 5. The plasma concentration reached $C_{max}$ (2328.1 ng/ml) 4 h after oral administration and decreased with a $t_{1/2}$ of 4.7 h. The $t_{1/2}$ for intravenous administration was 3.9 h; the plasma concentrations at 24 h after oral and intravenous administration were below the detection limit (10 ng/ml). Bioavailability was calculated as 65.2%, using the AUCs corrected for the doses given by oral and intravenous administration (Table 2). The concentrations in the prostate increased slowly, the $C_{max}$ of 395.6 ng/g of tissue occurred at 8 h and decreased to 45.8 ng/g of tissue at 24 h after oral administration. After intravenous administration, the concentration in the prostate reached a $C_{max}$ of 217.9 ng/g of tissue after 1 h, then decreased to below the detection limit at 24 h.

Table 2
Pharmacokinetic parameters of Z-350 of rats

| Parameter | i.v. | p.o. |
|---|---|---|
| Dose (mg/kg) | 1 | 10 |
| $T_{max}$ (h) | – | 4 |
| $C_{max}$ (ng/ml) | – | 2328.1 |
| AUC (ng·h/ml) | 2891.5 | 18,908.4 |
| CL/$F$ (ml/min/kg) | 326.3 | 500.8 |
| $t_{1/2}$ (h) | 3.9 | 4.7 |
| $F$ (%) | – | 65.2 |

Data were calculated from the mean values.

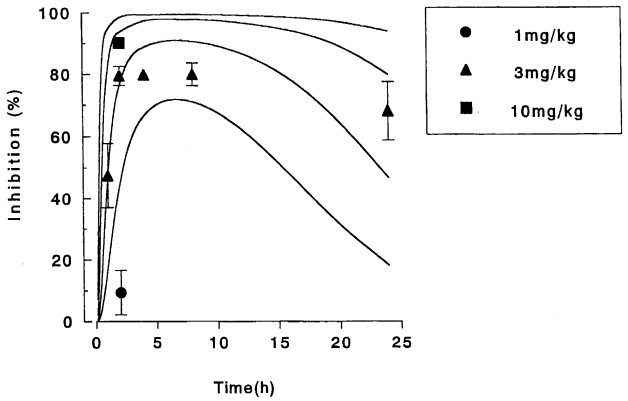


Fig. 6. Inhibition of 5α-reductase activity after a single oral administration of Z-350 to rats (1 mg/kg: circles, 3 mg/kg: triangles, 10 mg/kg: squares). Data are the mean ± S.E. values for five rats. The solid line shows the values at doses of 1, 3, 10 and 30 mg/kg, calculated using the pharmacodynamic model shown in Fig. 2.

The inhibition of 5α-reductase after oral administration of Z-350 is shown in Fig. 6. Z-350 appeared to inhibit 5α-reductase, from 1 h after oral administration at a dose of 3 mg/kg and the maximum inhibition was observed after 2–4 h, but the inhibitory effect was still detectable at 24 h after administration. The plasma and prostate concentration, and the inhibition of 5α-reductase at all doses, were simultaneously fitted to a pharmacodynamic model (Fig. 2), and the theoretical curves corresponded almost completely to the observed values at all doses.

Table 3
Pharmacokinetic and pharmacodynamic parameters of Z-350 in rats

| Parameter | | |
|---|---|---|
| $k_a$ | (1/h) | 0.150 |
| $k_{12}$ | (1/h) | 0.154 |
| $k_{21}$ | (1/h) | 0.098 |
| $k_{10}$ | (1/h) | 0.542 |
| $V_1$ | (l/kg) | 672.4 |
| $V_2$ | (l/kg) | 322.6 |
| $Q_{pr}$ / $V_3$ | (1/h) | 0.102 |
| $K_p$ | | 0.27 |
| $E_{max}$ | (%) | 100 |
| $n_H$ | | 1.25 |
| $EC_{50}^{n_H}$ | | 30.05 |

Table 4
Concentrations of Z-350 in plasma and prostate and 5α-reductase inhibition after multiple oral administration to rats (dose: 3 mg/kg/day)

| | | Concentration | | 5α-Reductase inhibition (%) |
|---|---|---|---|---|
| | | Plasma (ng/ml) | Prostate (ng/g of tissue) | |
| 1 day | (2 h) | 571.2 ± 47.0 | 128.9 ± 14.5 | 65.0 ± 7.0 |
| | (24 h) | N.D. | 28.4 ± 4.2 | 25.6 ± 12.0 |
| 4 days | (2 h) | 588.0 ± 68.3 | 351.6 ± 109.8 | 89.8 ± 2.2 |
| | (24 h) | N.D. | 96.5 ± 38.3 | 54.8 ± 13.6 |
| 7 days | (2 h) | 747.6 ± 108.4 | 238.0 ± 25.0 | 70.5 ± 3.2 |
| | (24 h) | N.D. | 96.3 ± 15.9 | 37.2 ± 6.0 |
| 14 days | (2 h) | 485.8 ± 30.2 | 173.4 ± 28.2 | 75.8 ± 1.3 |
| | (24 h) | N.D. | 40.7 ± 3.4 | 43.4 ± 8.1 |

Data are the means ± S.E. from three to five animals.
N.D.: not detected.

The pharmacodynamic parameters are shown in Table 3, $k_p$, $n_H$ and $EC_{50}^{n_H}$ were 0.27, 1.25 and 30.1 ng/g of tissue, respectively.

### *3.2. Plasma and prostate concentrations, and 5α-reductase inhibition actions after multiple administration*

The plasma and prostate concentrations and 5α-reductase inhibition at 2 and 24 h after multiple daily oral administration of Z-350 to rats at a dose of 3 mg/kg/day for 14 days are shown in Table 4. The plasma concentrations of Z-350 at 2 h after administration were almost at a constant level on the 1st, 4th, 7th and 14th days of treatment, and those at 24 h on all these days were below the detection limit. The prostate concentrations of Z-350 at 2 and 24 h after administration were almost constant on all these days. Z-350 apparently inhibited 5α-reductase 2 h after each administration; however, these effects diminished after 24 h on these days. The pharmacological parameters were obtained from the Hill equation; $n_H$ and $EC_{50}^{n_H}$ were 1.05 and 49.7 ng/g of tissue, respectively (Fig. 4B).

## 4. Discussion

Plasma and prostate concentrations 2 h after oral administration of 1, 3, 10 and 30 mg/kg of Z-350 increased almost in parallel with the dose. The $k_p$ value was calculated to be 0.25 by linear regression analysis of the prostate and plasma concentrations. Generally, carboxyl compounds have a high plasma protein binding ratio and the tissue concentration of these compounds is lower than the plasma concentration (Kurz and Fichtl, 1983). However, Z-350 could be distributed easily to the prostate because of its high lipophilicity in spite of its butyric acid moiety. In rats, two steroid 5α-reductase isozymes are encoded by two separate genes (Span et al. 1996). Z-350 inhibits 5α-reductase in hepatic and epididymal tissue, which express predominantly the type-1 and -2 isozymes, respectively (Fukuda et al., 1999). Therefore, Z-350 would be a subtype non-selective inhibitor of the rat enzymes. The inhibition of 5α-reductase by Z-350 was enhanced in proportion to the dose. Z-350 inhibits prostatic 5α-reductase, which results in a reduction of prostate enlargement (Fukuda et al., 2001). The measurement of Z-350 concentration in the prostate and the inhibition of 5α-reductase, calculated using the Hill equation, yielded values for $n_H$ of almost 1.0 and for $EC_{50}^{n_H}$ of 47.4 ng/g of tissue. In an in vitro study, Z-350 inhibited rat 5α-reductase with an $IC_{50}$ value of 8.42 nM (Fukuda et al., 1999). In this study, we calculated the $IC_{50}$ to be 1.2 nM, using the $EC_{50}^{n_H}$ value corrected for the protein concentration; this value was very similar to the in vitro $IC_{50}$ value. In addition, the plasma and prostate concentrations of Z-350 did not change during the period of multiple administration, which indicated that it did not accumulate. The extent of inhibition of 5α-reductase at 2 and 24 h was almost constant among the multiple administrations, and the pharmacological parameters, $n_H$ and $EC_{50}^{n_H}$ values obtained from the Hill equation, corresponded to those calculated for a single administration. The major metabolite of Z-350 was found to be a glucuronide conjugate at butyric acid terminals; other metabolites were aromatic hydroxylated metabolites. However, hydrolytic removal or cleavage metabolites could not be detected (unpublished data). These data indicate that Z-350 is distributed to its target organ, the prostate, and that it inhibits 5α-reductase in an unchanged form. In addition, the results of in vitro studies showed that Z-350 inhibited 5α-reductase reversibly in a non-competitive manner (Fukuda et al., 1999). Z-350 seemed to inhibit the 5α-reductase enzyme with a ratio of 1:1, because $n_H$ was calculated as being almost 1.

The plasma concentration of Z-350 reached $C_{max}$ at 4 h after oral administration of a dose of 10 mg/kg, and it was eliminated slowly with a $t_{1/2}$ of about 4.7 h. The $t_{1/2}$ was about 3.9 h after intravenous administration and the bioavailability was calculated to be 65.2%. The $T_{max}$ of Z-350 in the prostate was delayed compared with that in plasma and depended on the plasma flow rate. In addition, Z-350 in the prostate was eliminated slowly and was still detectable at 24 h; elimination from the prostate depended

on the plasma flow rate. After oral administration at a dose of 3 mg/kg, the inhibition of 5α-reductase reached a maximum at 4 h and was still observed at 24 h. We analyzed the plasma and prostate concentrations and inhibition of 5α-reductase simultaneously at several doses, using a pharmacodynamic model (Fig. 2). The theoretical curves for the plasma and prostate concentrations and the inhibition of 5α-reductase at several doses were in good agreement with the observed data (Figs. 5 and 6). The $k_p$ value (0.27; calculated by a pharmacodynamic model) corresponded to that calculated by linear regression. From these data, the disposition of Z-350 in rats followed linear kinetics at doses of 1–30 mg/kg. We simulated the inhibition of 5α-reductase by using prostate concentrations with the Hill equation, and estimated that the peak of inhibition was reached at doses higher than 10 mg/kg. In addition, the inhibition at 24 h after oral administration was greater than 50% at doses higher than 3 mg/kg. For the 1 mg/kg dose, inhibition was not detected at the first time point; however, we assumed that the inhibition might be greater than 50% from 4 to 8 h after administration.

In conclusion, the inhibition of 5α-reductase after the administration of Z-350 is dependent on the concentration of Z-350 in the prostate. The pharmacological response of Z-350 is maintained for 24 h after oral administration.

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