Prediction of $\alpha_1$-Adrenoceptor Occupancy in the Human Prostate from Plasma Concentrations of Silodosin, Tamsulosin and Terazosin to Treat Urinary Obstruction in Benign Prostatic Hyperplasia

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Abstract

$\alpha_1$-Adrenoceptor antagonists are clinically useful for the improvement of urinary obstruction due to benign prostatic hyperplasia (BPH), and their therapeutic effects are mediated through the blockade of prostatic $\alpha_1$-adrenoceptors. The present study was undertaken to predict the magnitude and duration of $\alpha_1$-adrenoceptor occupancy in the human prostate after oral $\alpha_1$-adrenoceptor antagonists. Prostatic $\alpha_1$-adrenoceptor-binding parameters of silodosin were estimated by measuring specific $[^3H]$prazosin binding in rat prostate after oral administration of this drug. The plasma concentration of silodosin after oral administration in rats and healthy volunteers was measured using a high-performance liquid chromatographic method. The $\alpha_1$-adrenoceptor-binding affinities ($K_i$) of silodosin, tamsulosin, and terazosin in the human prostate and plasma concentrations of tamsulosin and terazosin were obtained from the literature. Using the $\alpha_1$-adrenoceptor binding parameters of silodosin in rat prostate, $\alpha_1$-adrenoceptor occupancy in the human prostate was estimated to be around 60—70% at 1—6 h after oral administration of silodosin at doses of 3.0, 8.1, and 16.1 $\mu$mol. Thereafter, the receptor occupancy was periodically decreased, to 24% (8.1 $\mu$mol) and 54% (16.1 $\mu$mol) 24 h later. A similar magnitude and time course of $\alpha_1$-adrenoceptor occupancy by silodosin in the human prostate were estimated using $\alpha_1$-adrenoceptor-binding affinities ($K_i$) in the human prostate. Despite about two orders of differences in the plasma unbound concentrations after clinically effective oral dosages of silodosin, tamsulosin, and terazosin, there was a comparable magnitude of prostatic $\alpha_1$-adrenoceptor occupancy by these drugs. In conclusion, the prediction of $\alpha_1$-adrenoceptor occupancy in the human prostate by $\alpha_1$-adrenoceptor antagonists may provide the rationale for the optimum dosage regimen of these drugs in the therapy of BPH.

Key words tamsulosin; terazosin; silodosin; prostate; $\alpha_1$-adrenoceptor occupancy

The prediction of optimal dosage regimens in humans is important for clinical studies in the development of novel drugs and also for the risk assessment of adverse effects. For this purpose, it may be useful to estimate receptor occupancy for drugs in human target organs using drug-receptor-binding parameters and pharmacokinetic parameters. Several investigators, based on the receptor occupancy theory, have previously assessed the rational dosage, extent, and duration of pharmacologic effects, and the adverse effects of antagonists for histamine receptors, $\beta$-adrenoceptors, dopamine receptors, and muscarinic receptors in humans.1—4) Thus the receptor occupancy theory-based analysis in humans appears useful not only for estimating a rationale dosage regimen but also for determining the standard dose of new drugs using experimental data obtained in preclinical studies.

$\alpha_1$-Adrenoceptor antagonists are clinically useful for the improvement of urinary obstruction due to benign prostatic hyperplasia (BPH), and their pharmacologic effect is mediated through the blockade of prostatic $\alpha_1$-adrenoceptors.5—7) Tamsulosin and terazosin are in clinical use for the treatment of urinary obstruction in BPH. Silodosin (KMD-3213) has been shown to be a highly selective antagonist of the $\alpha_1A$-adrenoceptor subtype with prostatic selectivity,8—11) and this drug is now clinically used as a therapeutic agent for urinary outlet obstruction in patients with BPH. Although prostatic $\alpha_1$-adrenoceptor binding by $\alpha_1$-adrenoceptor antagonists has been characterized in vitro and in vivo,11—13) the occupancy by these drugs of $\alpha_1$-adrenoceptors in human prostate is not clear. The aim of the present study was to estimate time of occupancy by silodosin, tamsulosin, and terazosin of prostatic $\alpha_1$-adrenoceptors from the plasma unbound concentration after oral administration of these drugs at therapeutic doses in humans and from their $\alpha_1$-adrenoceptor-binding parameters to simulate the magnitude and duration of the clinical effects of these drugs.

MATERIALS AND METHODS

Materials $[^3H]$Prazosin (3.03 TBq/mmol) was purchased from Dupont-NEN Co., Ltd. (Boston, MA, U.S.A.). Silodosin was donated by Kissei Pharmaceutical Company (Matsusoto, Japan). All other chemicals were purchased from commercial sources.

Study Population and Protocol in Healthy Volunteers

Eighteen healthy male volunteers (six per dose), as determined by normal medical history, physical examination, and clinical laboratory parameters, participated in the study (age, 21.8±1.0 years; body weight, 62.9±7.7 kg, height, 172.5±6.3 cm). All volunteers gave written consent after they were informed of the aims of the trial. The protocol was reviewed and approved by the Ethics Committee of the Kitazato Institute, Research Center for Clinical Pharmacology, Japan. The trial was performed in accordance with Good Clinical Practice and the Guidelines of the International Conference on Harmonization. In the fasting condition, each volunteer received single oral doses silodosin of 3.0, 8.1, and 16.1 $\mu$mol. Blood samples for the high-performance liquid chromatographic (HPLC) assay of the plasma concentration of silodosin were collected 15 min—48 h after dosing.

Drug Administration of Silodosin in Rats

Male

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Sprague-Dawley rats weighing about 250—350 g were purchased from Japan SLC Inc. (Shizuoka, Japan). This study was conducted according to guidelines approved by the Experimental Animal Ethical Committee of the University of Shizuoka.

Rats received oral silodosin at pharmacologically relevant doses (0.6—20.2 μmol/kg) through a gastric tube. At 0.5—24 h after the drug administration, the rats were killed by exsanguination from the descending aorta under light anesthesia with diethyl ether, and perfused with 0.9% saline from the aorta. Then, the prostate was dissected and plasma was separated from the blood by centrifugation and stored at -20 °C.

**α1-Adrenoceptor Binding Assay** As previously described, the homogenate of rat prostatic tissue was incubated with various concentrations of [3H]prazosin in Tris–HCl buffer 50 mM for 30 min at 25 °C. The reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters. Tissue-bound radioactivity was determined using a liquid scintillation counter. Specific [3H]prazosin binding was determined experimentally from the difference between counts in the absence and presence of phentolamine 10 μM.

**Measurement of Plasma Concentration of Silodosin** The concentration of silodosin in rat plasma was determined using HPLC method. Briefly, standard solution, saturated sodium bicarbonate solution, and diethyl ether/dichloromethane were added to the plasma, and the mixture was shaken and centrifuged. The organic layer was transferred to a test tube and samples were subjected to HPLC method. The plasma unbound concentration of silodosin in rats was calculated using the free fraction (20.5%) from *in vitro* plasma protein binding of [3H]silodosin, and that in humans was 3.8%. The lower limit of determination of silodosin in this method was 0.2 nM in plasma, which corresponds to 0.04 nM of the plasma free concentration.

**Estimation of α1-Adrenoceptor Occupancy and Data Analysis** As described previously, the apparent dissociation constant (Kd) and maximum number of binding sites (Bmax) for [3H]prazosin were estimated in Rosenthal analysis. α1-Adrenoceptor occupancy (RO, %) in the rat prostate after oral administration of silodosin was calculated with the following equation:

\[
RO(\%) = \left( \frac{B_{\text{max(control)}} - B_{\text{max(silodosin)}}}{B_{\text{max(control)}}} \right) \times 100
\]

where \(B_{\text{max(control)}}\) and \(B_{\text{max(silodosin)}}\) are values for specific [3H]prazosin binding in the prostates of vehicle- and silodosin-treated rats, respectively.

Prostatic α1-adrenoceptor occupancy from the plasma unbound concentration of silodosin in rats was estimated with the following equation by means of nonlinear least-squares regression analysis using MULTI:

\[
RO(\%) = \left( \frac{RO_{\text{max}} \times C_i(\text{rat})}{\left[ C_i(\text{rat}) + OC_{50} \right]} \right) \times 100
\]

where \(RO_{\text{max}}\) = maximum occupancy of α1-adrenoceptors; \(OC_{50}\) = plasma unbound concentration of silodosin necessary for 50% occupancy of prostatic α1-adrenoceptors; and \(C_i(\text{rat})\) = rat plasma unbound concentration of silodosin.

Prostatic α1-adrenoceptor occupancy in human prostates was estimated from plasma unbound concentrations of silodosin, tamsulosin, and terazosin in healthy volunteers with the following equations:

\[
RO(\%) = \left( \frac{RO_{\text{max}} \times C_i(\text{human})}{\left[ C_i(\text{human}) + OC_{50} \right]} \right) \times 100
\]

\[
RO(\%) = \left( \frac{C_i(\text{human})}{C_i(\text{human}) + K_i} \right) \times 100
\]

where \(C_i(\text{human})\) = human plasma unbound concentration of α1-adrenoceptor antagonists; and \(K_i\) = inhibition constant for α1-adrenoceptor antagonists.

In the case of silodosin, there were two binding sites consisting of high- \((K_i = 0.042 \text{ nM}, \text{ fraction} = 66\%)\) and low- \((K_i = 15 \text{ nM}, \text{ fraction} = 34\%)\) affinity sites in the human prostate. The following equation was used to estimate prostatic α1-adrenoceptor occupancy.

\[
RO(\%) = \left( \frac{F_h \times C_i(\text{human})}{C_i(\text{human}) + K_i(\text{high})} + F_l \times C_i(\text{human}) \times K_i(\text{low}) \right) \times 100
\]

where \(F_h\) = fraction of high-affinity site for silodosin in the human prostate; \(F_l\) = fraction of low-affinity site for silodosin in the human prostate; \(K_i(\text{high})\) = inhibition constant for silodosin at high-affinity site; and \(K_i(\text{low})\) = inhibition constant for silodosin at low-affinity site.

**RESULTS**

**Estimation of α1-Adrenoceptor Occupancy Based on α1-Adrenoceptor-Binding Parameters in Rat Prostate** \(K_d\) and \(B_{\text{max}}\) values for specific [3H]prazosin binding in rat prostatic tissues were 0.068 ± 0.010 nM, and 30.5 ± 5.0 fmol/mg protein (mean ± S.E., n = 9), respectively. Oral administration of silodosin (0.6—20.2 μmol/kg) in rats resulted in a significant decrease in the \(B_{\text{max}}\) value for prostatic [3H]prazosin binding with little effect on \(K_d\). As shown in Fig. 1, prostatic α1-adrenoceptor occupancy by silodosin which was calculated from the decrease in \(B_{\text{max}}\) for [3H]prazosin (Eq. 1) reached a plateau at 1 nM. From the calculation of Eq. 2,

![Fig. 1. α1-Adrenoceptor Occupancy in the Prostate as a Function of the Increase in Plasma Unbound Concentrations of Silodosin after Oral Administration at Doses of 0.6, 2.0, 6.1, and 20.2 μmol/kg in Rats](image-url)
Plasma unbound concentrations of silodosin in healthy volunteers were dose dependently increased 0.25—24 h after oral administration of this drug at 3.0, 8.1, and 16.1 \( \mu \text{mol} \) (Fig. 2). The concentration of silodosin at each dose peaked about 1 h after oral administration and decreased gradually until approximately 5 h. From plasma unbound concentrations of silodosin in healthy volunteers, \( \alpha_1 \)-adrenoceptor occupancy in the human prostate was estimated with Eq. 3 using \( \alpha_1 \)-adrenoceptor binding parameters in rat prostate. As shown in Fig. 3, prostatic \( \alpha_1 \)-adrenoceptor occupancy was consistently around 60—70\% 1—6 h after oral administration of silodosin at doses of 3.0—16.1 \( \mu \text{mol} \), and 12 h later, the receptor occupancy was 50—70\%. Respective occupancy 24 h later was decreased to 7\% (3.0 \( \mu \text{mol} \)), 24\% (8.1 \( \mu \text{mol} \)), and 54\% (16.1 \( \mu \text{mol} \)).

**Estimation of \( \alpha_1 \)-Adrenoceptor Occupancy by \( \alpha_1 \)-Adrenoceptor-Binding Parameters in the Human Prostate**

\( \alpha_1 \)-Adrenoceptor occupancy in the human prostate was estimated using Eq. 4 using \( \alpha_1 \)-adrenoceptor-binding affinities of silodosin in the human prostate and plasma unbound concentration of silodosin after oral administration (Fig. 2). As shown in Fig. 4, the maximum plasma unbound concentration of tamsulosin (4 h) after oral administration (slow-release dosage form: 0.45 \( \mu \text{mol} \)) in healthy volunteers was 0.16 nM and the plasma unbound concentration 24 h later was 0.03 nM. Similarly, the maximum plasma unbound concentration of terazosin after oral administration (2.2 \( \mu \text{mol} \)) was 9.51 nM and 24 h later it was 1.22 nM. From these data, \( \alpha_1 \)-adrenoceptor occupancy by both drugs in the human prostate was estimated using \( \alpha_1 \)-adrenoceptor-binding affinities (\( K_i \) for tamsulosin = 0.04 nM, \( K_i \) for terazosin = 1.04 nM) in the human prostate. The peak values of prostatic \( \alpha_1 \)-adrenoceptor occupancy by tamsulosin and terazosin were 80\% (4 h) and 90\% (1 h), respectively, and the lowest values 24 h later were 44\% and 54\%, respectively (Fig. 5).

**DISCUSSION**

\( \alpha_1 \)-Adrenoceptor occupancy in the human prostate was predicted from plasma unbound concentrations after a single oral administration of clinically effective dosages of silodosin, tamsulosin, and terazosin in healthy volunteers. \( \alpha_1 \)-Adrenoceptor binding affinities (\( pK_i \) of \( \alpha_1 \)-adrenoceptor antagonists in the rat prostate) correlated significantly with those in the human prostate. Therefore \( \alpha_1 \)-adrenoceptor binding parameters in rat prostate might be applicable for the simulation of \( \alpha_1 \)-adrenoceptor occupancy in the human prostate. There was a dose-dependent increase in the plasma unbound concentration of silodosin in healthy volunteers.
Thereafter, the receptor occupancy decreased with time and after oral administration of silodosin at three different doses. The human prostate was estimated to be around 60—70% 1—6 h after oral administration of silodosin at doses of 0.45, 2.2, and 16.1 μmol, respectively, in healthy volunteers.

The inhibitory potencies of silodosin, terazosin, prazosin, and tamsulosin were estimated by using specific [3H]prazosin binding. The inhibition by silodosin of specific [3H]prazosin binding in rat prostate was 9.46 (our unpublished data). These data coincide well with previous reports describing a relatively long duration of pharmacologic effects in rat prostate. Consequently, the prediction of prostatic α1-adrenoceptor occupancy in the present study suggests a relatively long duration of the clinical effects of silodosin in patients with BPH.

Furthermore, based on Ki values for competitive inhibition of human prostate α1-adrenoceptor radioligand binding and plasma unbound concentrations in healthy volunteers, α1-adrenoceptor occupancy in the human prostate by tamsulosin and terazosin at clinical doses was estimated to be 70—90% 1—6 h after oral administration, and 40—50% even 24 h later. Long-lasting occupancy of prostatic α1-adrenoceptors was also demonstrated in in vivo experiments with silodosin in rats. These data coincide well with previous reports describing a relatively long duration of pharmacologic effects in rat prostate. Consequently, the prediction of prostatic α1-adrenoceptor occupancy in the present study suggests a relatively long duration of the clinical effects of silodosin in patients with BPH.

Our preliminary study has shown that α1-adrenoceptor occupancy by approximately 20% of α1-adrenoceptors in rat prostate may be consistent among these drugs.

Fig. 5. Time Courses of Plasma Unbound Concentration (a) and Prostatic α1-Adrenoceptor Occupancy Predicted from Ki Values in Human Prostate (b) after Oral Administration of Tamsulosin (Δ), Terazosin (○), and Silodosin (□) at Clinical Doses of 0.45, 2.2, and 16.1 μmol, Respectively, in Healthy Volunteers

Each point represents the value estimated from average plasma concentration of each drug and estimated value of α1-adrenoceptor occupancy.

Fig. 6. Correlation between Rat Prostate and the Human Prostate in in Vitro Inhibitory Potencies (pKi) by α1-Adrenoceptor Antagonists (Silodosin, Terazosin, Prazosin, and Tamsulosin) of Specific Binding of α1-Adrenoceptor Radioligands ([3H]Prazosin, [3H]Tamsulosin, and [125I]HEAT)

Each point corresponds to the pKi value as follows: 1: pKi for the inhibition by silodosin of specific binding of [3H]prazosin in rat prostate and [125I]HEAT in human prostate. 2: pKi for the inhibition by terazosin of specific [3H]prazosin binding. 3: pKi for the inhibition by prazosin of specific [3H]tamsulosin binding. 4: pKi for the inhibition by tamsulosin of [3H]tamsulosin binding. 5: pKi for the inhibition by tamsulosin of specific [3H]prazosin binding. pKi values in rat prostate were cited from Okkura et al. and Shibata et al. The pKic values for the inhibition by prazosin and tamsulosin of specific [3H]tamsulosin in rat prostate were 9.89 and 10.4, respectively, and the pKi value for the inhibition by silodosin of specific [3H]prazosin binding in rat prostate was 9.46 (our unpublished data). The points fit the equation y = 0.83x + 1.28, r = 0.89 (p < 0.05), where y, x, and r are pKi values in the human prostate, pKi values in rat prostate, and correlation coefficient, respectively.

Volunteers after oral administration of silodosin at doses of 0.3, 8.1, and 16.1 μmol. Using α1-adrenoceptor-binding parameters in rat prostate, α1-adrenoceptor occupancy in the human prostate was estimated to be around 60—70% at the peak time (1—6 h) and that the duration of α1-adrenoceptor occupancy by silodosin is largely dependent on the oral dosage. Therefore, the magnitude and time course of α1-adrenoceptor occupancy by silodosin estimated using Ki values in the human prostate were comparable to those estimated using α1-adrenoceptor-binding parameters in rat prostate after oral administration of this drug. α1-Adrenoceptor occupancy ranged from 60—70% 1—2 h after oral administration of silodosin 16.1 μmol, thereafter decreasing gradually with time, and the receptor occupancy was approximately 40—50% even 24 h later. Long-lasting occupancy of prostatic α1-adrenoceptors was also demonstrated in in vivo experiments with silodosin in rats. These data coincide well with previous reports describing a relatively long duration of pharmacologic effects in rat prostate. Consequently, the prediction of prostatic α1-adrenoceptor occupancy in the present study suggests a relatively long duration of the clinical effects of silodosin in patients with BPH.

Furthermore, based on Ki values for competitive inhibition of human prostate α1-adrenoceptor radioligand binding and plasma unbound concentrations in healthy volunteers, α1-adrenoceptor occupancy in the human prostate by tamsulosin and terazosin at clinical doses was estimated to be 70—90% 1—6 h after oral administration, and 40—50% even 24 h later. Thus it should be noted that, despite about two orders of differences in plasma unbound concentrations after clinically effective oral dosages of silodosin, tamsulosin and terazosin, there may be comparable magnitude of prostatic α1-adrenoceptor occupancy after oral administration of these drugs in humans. This finding indicates that prostatic α1-adrenoceptor occupancy after oral administration of therapeutic doses of α1-adrenoceptor antagonists may be consistent among these drugs.

Our preliminary study has shown that α1-adrenoceptor occupancy by approximately 20% of α1-adrenoceptors in rat prostate may be the threshold value for exerting a significant attenuation of phenylephrine-induced increase in the intraurethral pressure based on the blockade of prostatic α1-adrenoceptors. Considering the prediction that prostatic α1-adrenoceptor occupancy would be more than 40% 12 and/or 24 h after oral administration of silodosin (8.1 μmol × 2/d), tamsulosin (0.45 μmol/d), and terazosin (1.1 μmol × 2/d) at
clinically used doses, it is expected that beneficial effects of these drugs for the treatment of urinary obstruction in patients with BPH may persist even 24 h after oral administration at clinical doses. Silodosin at this dose regimen (16.1 μmol/d) and tamsulosin (0.45 μmol) in the once-daily dosage form have been shown to be pharmacologically effective for the treatment of urinary obstruction with BPH. It is notable that silodosin, tamsulosin, and terazosin in clinically used dose regimens showed a roughly similar extent and duration of $\alpha_1$-adrenoceptor occupancy in the human prostate in the present prediction procedure.

In conclusion, the present study showed that the prediction of $\alpha_1$-adrenoceptor occupancy in the human prostate by silodosin, tamsulosin, and terazosin may provide the rationale for the dosage regimen of these drugs in the therapy of BPH. Thus it could be useful to predict appropriate dosage regimens of novel $\alpha_1$-adrenoceptor antagonists based on the simulation of $\alpha_1$-adrenoceptor occupancy in the human prostate using their $\alpha_1$-adrenoceptor-binding parameters and plasma unbound concentrations.

REFERENCES