Relationship between the Functional Effect of Tamsulosin and Its Concentration in Lower Urinary Tract Tissues in Dogs

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We investigated the relationship between the pharmacological effect of tamsulosin and its concentrations in plasma and several lower urinary tract (LUT) and arterial tissues in conscious male dogs. Oral administration of tamsulosin (30 and 100 μg/kg) inhibited phenylephrine-induced intraurethral pressure (IUP) elevation. Inhibition peaked at 1, 2 h after dosing and lasted up to 6—8 h. Basal mean blood pressure did not significantly change throughout the observation period. Plasma concentration reached maximum within 0.5 h after dosing, whereas that in LUT tissues (prostate, urethra and bladder) reached maximum at 1, 2 h, and prostatic and urethral concentrations remained higher than those in plasma and arterial tissues at almost all observation points. Prostatic concentrations of tamsulosin at individual time points were 2.2—18.0-fold higher than plasma and 3.7—12.3-fold higher than mesenteric artery concentrations. Urethral concentrations of tamsulosin were also higher than those in both plasma and mesenteric artery. The prostatic and urethral concentrations of tamsulosin correlated well with its effect on IUP response (r²=0.98 (p<0.01) and r²=0.99 (p<0.01), respectively). Our data demonstrate that tamsulosin is selectively retained in LUT tissues compared with plasma and arterial tissues and that its sustained effect on IUP response appears to be related to the prostatic and urethral retention of tamsulosin.

Key words tamsulosin; urethral pressure; tissue concentration; prostate; retention

The pathophysiology of bladder outlet obstruction (BOO) in men with benign prostatic hyperplasia (BPH) has been attributed to both static and dynamic factors.1 Static obstruction arises from adenoma enlargement of the prostate, whereas dynamic obstruction is related to an elevation in prostatic and urethral smooth muscle. Because this latter effect is predominantly related to local α-adrenergic tone, α1-adrenoceptor antagonists are used to relax prostatic and urethral smooth muscle, and thereby ameliorate lower urinary tract symptoms (LUTS) such as voiding symptoms (e.g., slow stream, hesitancy, intermittency, terminal dribble) and storage symptoms (e.g. increased daytime frequency, nocturia, urgency).2,3

Molecular and pharmacological studies have led to the classification of three α1-adrenoceptor subtypes: α1A, α1D and α1D-adrenoceptors.4,5 The α1A-adrenoceptor subtype has been described as predominant in the human prostate and urethra,6,7 and plays a predominant role in mediating the contractile response of the human prostate.8

Tamsulosin is an α1-adrenoceptor antagonist developed primarily for the treatment of LUTS suggestive of BOO.9—11 In radioligand binding assays, tamsulosin showed greater than 10-fold selectivity for α1A over α1D-adrenoceptors, with intermediate affinity for α1D-adrenoceptors.12—14 Tamsulosin also shows clinical uroselectivity in comparison with terazosin and doxazosin, α1-adrenoceptor antagonists originally developed as antihypertensive agents. Thus, tamsulosin has been shown to improve LUTS with minimal hypotensive effects and a low incidence of circulatory adverse events.15 Although it has been postulated that the α1A-selectivity of tamsulosin16 and its modified release formulation17 contribute to its uroselectivity, these hypotheses remain controversial.

Previously, we investigated the relationship between the pharmacokinetics of tamsulosin and its inhibitory effect on hypogastic nerve stimulation (HNS)-induced prostatic intrarethral pressure (IUP) elevation in anesthetized male dogs.18 Results showed that this inhibitory effect lasted up to 4 h without attenuation, despite the fact that the plasma concentration of tamsulosin had decreased to near the low limit of quantitation (LLOQ) at this time. Interestingly, prostatic and urethral concentrations were 13—44-fold higher than the plasma concentration at this time point, suggesting that the sustained effect of tamsulosin on the IUP response is related to its prostatic and urethral retention. To date, however, the time course of LUT tissue concentration has not been investigated. In addition, interest has been shown in both the relationship between effects and LUT tissue concentrations of tamsulosin, as well as in its distribution in other tissues.

Here, to evaluate the relationship between the functional effect of tamsulosin and its concentration in LUT tissues, we investigated the effect of orally administered tamsulosin on phenylephrine (PE)-induced IUP response and the time course of concentrations in plasma and several LUT tissues in conscious male dogs. We also compared LUT tissue concentrations with those in arterial tissues.

MATERIALS AND METHODS

Functional Experiment The animal experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical (premerge name of Astellas Pharma Inc.). Male beagle dogs (9—15.5 kg) were fasted overnight and anesthetized with thiopental sodium (20 mg/kg i.v.). After endotracheal intubation, the animals were spontaneously ventilated with room air and a catheter sheath (RS-A50K10A, Terumo, Japan) was placed in the femoral artery. For 1 to 4 d after operation, a dog was placed in a sling restraint and blood pressure was measured with a pressure amplifier (AP-641G, Nihon Kohden, Tokyo, March 2007


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Japan) via a pressure transducer (TP-400T, Nihon Kohden) connected to a 5 Fr Swan-Ganz thermodilution catheter (TC-504, Nihon Kohden) placed in the aorta via the sheath inserted in the femoral artery. To record the prosthetic IUP, a modified thermodilution balloon catheter (6 Fr) was introduced into the bladder via the external urethral meatus, placed in the prostatic urethra according to the method of Brune et al., and then inflated with distilled water. The balloon port of the catheter was connected to a pressure transducer (TP-400T, Nihon Kohden). Urine in bladder was discharged via a hole in the point of the balloon catheter to eliminate the possible effect of residual urine on urethral pressure. Intraarterial administration of PE (5 μg/kg, 0.02 ml/kg) was done via the catheter inserted into the lower portion of the abdominal aorta. After stabilization of the urethral pressure response induced by PE, tamsulosin (0, 30, 100 μg/kg, 1 ml/kg) was administered orally, followed by PE challenge at 0.5, 1, 2, 4, 6, 8 and 24 h after dosing. Heparinized blood samples were obtained from the femoral artery of each dog at 5, 10, 15 and 30 min and 1, 2, 4, 6, 8 and 24 h after dosing. Plasma was separated by centrifugation (4°C). Dogs receiving 100 μg/kg of tamsulosin were euthanized by pentobarbital overdose immediately after measurement of the final IUP response and the prostate, proximal urethra, bladder base, bladder body, carotid artery, aorta and mesenteric artery were excised. The plasma and tissue samples were frozen in liquid nitrogen and stored at −80°C until analysis.

**Time Course of Tissue Concentrations** Male beagle dogs (9.5—15 kg) were orally administered tamsulosin (30, 100 μg/kg) after an overnight fast. The prostate, proximal urethra, bladder base, bladder body, carotid artery, aorta and mesenteric artery were excised immediately after euthanization by pentobarbital overdose at 0.5 (at 30 μg/kg), 1, 2, 4, 6 and 8 h after tamsulosin dosing and frozen in liquid nitrogen. Heparinized blood samples were obtained from the cephalic vein of each dog at the same time as tissue sampling. Plasma was separated from the red cells by centrifugation (4°C). The plasma and tissue samples were stored at −80°C until analysis.

**Determination of Plasma and Tissue Concentrations** Determination of unchanged tamsulosin in plasma and several tissues was performed by a modified version of the procedure of Soeishi et al., in combination with LC-MS/MS at the Main Reference Laboratory of Mitsubishi Kagaku Bio- Clinical Laboratories Inc. (Tokyo, Japan). LLOQ were 0.05 ng/ml for plasma and 0.25 ng/g for tissues, respectively.

\[ (+/-)-(R)-5-[3-[2-(O-Ethoxy-phenoxy)ethyl]amino]-2-methoxy-benzensulfonylamide hydrochloride (AB289, Lot No. T-4912) supplied by Yamanouchi Pharmaceutical Co., Ltd., was used as the internal standard.

**Calculation of Pharmacokinetic Parameters** In the functional experiment, plasma concentration was analyzed by the model-independent method. The maximal concentration (C_max) of plasma and time to C_max (T_max) were observed values and the area under the concentration–time curve (AUC_lag) of plasma was calculated using the log-linear trapezoidal method. Elimination half-life (t_1/2) was determined by least squares regression analysis of the terminal log-linear portions of the plasma concentration–time profile. In the time course of the tissue concentration experiment, C_max and T_max of tissues and plasma were the mean values of 4 animals and AUC_lag was analyzed using the log-linear trapezoidal method.

**Drugs** Tamsulosin hydrochloride was synthesized at Yamanouchi Pharmaceutical Co., Ltd. Phenytoephline hydrochloride (Neo-Synephrine) and thiopental sodium (Ravalon) were purchased from Kowa Pharmaceutical Co., Ltd. (Tokyo, Japan) and Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Tamsulosin was dissolved and diluted with distilled water. Phenytoephine hydrochloride was diluted with saline.

**Statistical Analysis** The degree of inhibition of PE-induced IUP response and effect on mean blood pressure (MBP) at each time point during the time course of the experiment were compared using two-way repeated measures ANOVA followed by Dunnett’s multiple range test. Differences were considered significant at p<0.05. The relationship between efficacy and tamsulosin concentration was analyzed by plotting the time course inhibition of IUP response with the plasma, prostate and urethra concentrations. The relationship between the inhibition of IUP response and LUT tissue concentration was analyzed by linear regression analysis.

**RESULTS**

**Functional Experiment** In conscious dogs, IUP elevation could be elicited by intraarterial injection of PE into the lower portion of the abdominal aorta. PE produced almost no effect on basal MBP, indicating PE was likely selectively distributed to the lower urinary tract. As shown in Fig. 1, PE induced reproducible and submaximal increases in IUP over 24 h, with no statistical differences seen in predosing IUP responses between experimental groups. Figure 1 shows the
time course of tamsulosin effects on basal MBP and PE-induced increases in prostatic IUP in conscious dogs. Tamsulosin (30, 100 mg/kg p.o.) inhibited PE-induced IUP elevation with maximal effects at 1 and 2 h after dosing, respectively, and the inhibition lasted up to 6 and 8 h at 30 and 100 mg/kg p.o., respectively. Tamsulosin caused a slight but not significant decrease in basal MBP.

**Plasma Concentrations** Figure 2 shows the time course of the plasma concentration of tamsulosin after oral administration of Tamsulosin (30 µg/kg) in conscious dogs. Each point represents the mean±S.E.M. of 5 animals. Table 1 lists pharmacokinetic parameters for individual animals in each dose group. Plasma concentrations reached maximum (C<sub>max</sub>: 11.4±1.9 and 15.4±6.7 ng/ml) at 0.2 and 0.4 h after dosing and declined with t<sub>1/2</sub> values of 1.1 and 1.6 h, respectively, while AUC<sub>last</sub> values were 11.8 and 25.4 ng·h/ml, respectively. Concentrations had declined below the LLOQ at 24 h after dosing.

**Tissue Concentrations** Figures 3 and 4 show the time course of various LUT tissues, arterial and plasma concentrations of tamsulosin, and Tables 2 and 3 list pharmacokinetic parameters after oral administration at 30 and 100 µg/kg, respectively. At 30 µg/kg, LUT tissue (prostate, urethra, and bladder) concentrations reached maximum at 1 h after administration, and prostatic and urethral concentrations remained higher than those in plasma and arterial tissues at almost all observation points. The rank order of AUC<sub>last</sub> was
Concentrations

The inhibitory effect of tamsulosin on IUP that in the bladder base and body (data not shown). 1h after dosing, when plasma concentration was higher than mesenteric artery during the observation period, except up to higher than that in mesenteric artery. Concentration in the fold higher than plasma concentration and 2.2- to 2.9-fold higher than that in mesenteric artery. Urethral concentration was 1.4- to 11.0-fold higher than plasma concentration and 3.8- to 6.6-fold higher than that in prostate. Prostatic concentration was 3.7- to 18.0-fold higher than plasma concentration and 2.2- to 4.2-fold higher than that in mesenteric artery. Concentration in the tissue concentration in Fig. 3 (30 g/kg, LUT last was 0.99 (p<0.01), respectively].

Relationship between Efficacy and Plasma and Tissue Concentrations

Prostate > urethra > aorta > bladder base > plasma > bladder body > mesenteric artery > carotid artery. At 100 μg/kg, LUT tissues concentrations reached maximum at 2 h after administration, and prostatic and urethral concentrations remained higher than those in plasma and arterial tissues throughout the observation period. The rank order of AUC last was prostate > urethra > bladder base > bladder body > mesenteric artery > plasma = aorta = carotid artery.

Tables 4 and 5 show the time course of the ratio of prostatic and urethral concentrations versus individual plasma and mesenteric artery concentrations. Prostate/plasma and urethra/plasma ratios were time-dependently increased at 0.5 to 6 h after dosing at 30 μg/kg. Prostatic concentration was 2.2- to 10.5-fold higher than plasma concentration and 3.7- to 12.3-fold higher than that in mesenteric artery. Urethral concentration was 0.8- to 6.6-fold higher than plasma concentration and 2.2- to 4.2-fold higher than that in mesenteric artery. After administration at 100 μg/kg, prostate/plasma and urethra/plasma ratios also time-dependently increased at 1 to 8 h. Prostatic concentration was 3.7- to 18.0-fold higher than plasma concentration and 3.8- to 6.6-fold higher than that in mesenteric artery. Urethral concentration was 1.4- to 11.0-fold higher than plasma concentration and 2.2- to 2.9-fold higher than that in mesenteric artery. Concentration in the bladder base and body was higher than in either plasma or mesenteric artery during the observation period, except up to 1 h after dosing, when plasma concentration was higher than that in the bladder base and body (data not shown).

**DISCUSSION**

Our study shows that tamsulosin is preferentially retained response is plotted against plasma tamsulosin concentration in Fig. 5A. The resulting curves exhibited a counterclockwise hysteresis loop. Figure 5B shows the inhibitory effect of tamsulosin on IUP response plotted against prostatic and urethral concentrations. Prostatic and urethral concentrations correlated well with the effect on IUP response \[r^2=0.98 \ (p<0.01) \] and \[r^2=0.99 \ (p<0.01), \] respectively.

**Table 2. Pharmacokinetic Parameters of Several Tissues and Plasma after Oral Administration of Tamsulosin Hydrochloride (30 μg/kg) in Conscious Dogs**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C_{max} (ng/ml)</th>
<th>T_{max} (h)</th>
<th>AUC_{last} (ng·h/ml or ng·h/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5.2 (1)^{\text{a}}</td>
<td>0.5</td>
<td>9.3 (1)^{\text{a}}</td>
</tr>
<tr>
<td>Prostate</td>
<td>13.2 (2.6)</td>
<td>1</td>
<td>42.4 (4.5)</td>
</tr>
<tr>
<td>Urethra</td>
<td>4.6 (0.9)</td>
<td>1</td>
<td>17.4 (1.9)</td>
</tr>
<tr>
<td>Bladder base</td>
<td>2.7 (0.5)</td>
<td>1</td>
<td>11.4 (1.2)</td>
</tr>
<tr>
<td>Bladder body</td>
<td>2.4 (0.5)</td>
<td>1</td>
<td>8.2 (0.9)</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>1.6 (0.3)</td>
<td>0.5</td>
<td>5.1 (0.5)</td>
</tr>
<tr>
<td>Aorta</td>
<td>2.4 (0.5)</td>
<td>2</td>
<td>14.2 (1.5)</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>1.2 (0.2)</td>
<td>1</td>
<td>5.9 (0.6)</td>
</tr>
</tbody>
</table>

Values are the means of 4 animals. a) Ratios of C_{max} and AUC_{last} of tissues against those of plasma are shown in parentheses.

**Table 3. Pharmacokinetic Parameters of Several Tissues and Plasma after Oral Administration of Tamsulosin (100 μg/kg) in Conscious Dogs**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C_{max} (ng/ml)</th>
<th>T_{max} (h)</th>
<th>AUC_{last} (ng·h/ml or ng·h/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>9.5 (1)^{\text{a}}</td>
<td>1</td>
<td>20.1 (1)</td>
</tr>
<tr>
<td>Prostate</td>
<td>30.9 (3.3)</td>
<td>2</td>
<td>117.4 (5.8)</td>
</tr>
<tr>
<td>Urethra</td>
<td>14.4 (1.5)</td>
<td>2</td>
<td>55.2 (2.7)</td>
</tr>
<tr>
<td>Bladder base</td>
<td>8.4 (0.9)</td>
<td>2</td>
<td>36.4 (1.8)</td>
</tr>
<tr>
<td>Bladder body</td>
<td>6.4 (0.7)</td>
<td>2</td>
<td>27.9 (1.4)</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>4.0 (0.4)</td>
<td>1</td>
<td>13.0 (0.6)</td>
</tr>
<tr>
<td>Aorta</td>
<td>5.4 (0.6)</td>
<td>1</td>
<td>19.2 (1.0)</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>5.2 (0.5)</td>
<td>2</td>
<td>25.3 (1.3)</td>
</tr>
</tbody>
</table>

Values are the means of 4 animals. a) Ratios of C_{max} and AUC_{last} of tissues against those of plasma are shown in parentheses.
in LUT tissues such as prostate and urethra rather than plasma and arterial tissues in conscious male dogs. Further, the time course of urethral and prostatic concentration shows a strong correlation with its pharmacological effect.

The plasma concentration of tamsulosin quickly increased after dosing with a T_{\text{max}} of 0.2—0.4 h, whereas its maximum inhibition of PE-induced IUP elevation occurred at 1, 2 h. When the inhibitory effect of tamsulosin on IUP response was plotted against plasma tamsulosin concentration, the resulting curves exhibited an anticlockwise hysteresis loop, indicating the presence of a time lag between plasma concentration and pharmacological effect.

A similar phenomenon was observed in a previous study in anesthetized dogs, in which the urethral effect peaked about 90 min after dosing and lasted for 240 min without attenuation, whereas the plasma concentration quickly increased with a T_{\text{max}} of 10—30 min and declined gradually thereafter.17) These observations indicated the possibility of an equilibration delay between plasma concentration and the compartment of the site of action. Further, an in vitro functional study suggested that equilibration of the effect of tamsulosin requires time in the human prostate but not in the rat spleen or rat aorta,21) indicating that the association of tamsulosin with prostatic and/or urethral α1 receptors, namely α1A adrenoceptors, may be later than that with α₁ adrenoceptors in other tissues.

In the present study, T_{\text{max}} of LUT tissue concentrations of tamsulosin were 1, 2 h, corresponding with the time of maximal effect on IUP response. Curves plotting the inhibitory effect on IUP response against prostatic and urethral concentration did not exhibit an anticlockwise hysteresis loop, but rather showed good linearity (r²=0.98 in prostate, and r²=0.99 in urethra). This observation suggests that the inhibitory effect of tamsulosin on IUP response correlates with LUT tissues rather than plasma concentration.

Moreover, while the urethral effect of tamsulosin was sustained for 6—8 h after dosing, the plasma concentration at this time had decreased to near the LLOQ level, indicating a separation between effect and plasma concentration in the elimination phase of tamsulosin also. The ratio of prostatic and urethral concentrations versus plasma concentration was 1.3- to 3.7-fold at 1 h after dosing. This ratio increased time dependently and reached 10.5- to 18-fold in the prostate and 1.3- to 3.7-fold at 1 h after dosing. This result indicated the sustained effect of tamsulosin on IUP response is related to its prostatic and urethral retention. In rats, tamsulosin showed sustained occupancy of α₁-adrenoceptors in the prostate after a marked reduction in plasma concentration.25)

One explanation for this retention of tamsulosin may be its characteristic binding kinetics action on the α₁ adrenoceptor subtype of prostate and urethra, namely α₁A adrenoceptors. In fact, an in vitro functional study suggested that equilibration of the effect of tamsulosin requires time in the human prostate (α₁A receptors) but not in the rat spleen (α₁B receptors) or rat aorta (α₁C receptors).21) In a binding assay using human prostatic membranes, the dissociation constant of [³H]tamsulosin was 5-fold slower than that of [³H]prazosin, a representative non-α₁ subtype-selective antagonist.23) This result suggests that tamsulosin may show slow dissociation kinetics on α₁A adrenoceptors. It is well known that tamsulosin acts as a competitive antagonist in the urethral and prostatic tissues.21,24) In our present study, the effect of tamsulosin on urethral pressure was completely reversed to pre-value level 24 h after dosing. These results suggest irreversible antagonism by tamsulosin can be ruled out.

To allow comparison with LUT concentrations, we also measured aorta, carotid and mesenteric artery concentrations of tamsulosin. Whereas AUC_{\text{max}} in prostate and urethra was 1.9- to 5.8-fold higher than that in plasma, values in these three arterial tissues were closely similar to that in plasma. In addition, calculation of the ratio of prostatic and urethral concentration of tamsulosin versus that in mesenteric artery, one of the resistance arteries regulating arterial pressure, gave ratios 3.7- to 12.3-fold greater in prostate and 2.2- to 4.2-fold greater in urethra, indicating that tamsulosin produces a selective and sustained distribution in prostate and urethra, the target organs for the treatment of BOO in patients with BPH. Although the mechanism of retention of tamsulosin in prostatic and urethral tissues than arterial tissues is unclear, we postulate that it may involve differences in α₁ receptor subtype and/or the amount of α₁ receptor expression between LUT tissues and arteries and/or preferential retention in these LUT tissue matrices. Further studies are warranted to elucidate its mechanism of action in detail.

In comparison with doxazosin and terazosin, which are non-α₁ subtype-selective antagonists, tamsulosin has a selective effect on urethral pressure over blood pressure in conscious dogs.25) In placebo-controlled clinical trials, tamsulosin has been reported to show a lower incidence of cardiovascular side effects than has been observed with doxazosin or terazosin.15) Although the reason for the uroselective profile of tamsulosin remains controversial, the slower absorption and decrease in C_{\text{max}} by the modified release oral formulation and α₁ subtype selectivity may be involved.11,16)

In our present study, prostatic and urethral concentrations of tamsulosin were higher at each time point than both plasma and vascular concentrations, suggesting that the selective distribution and retention of tamsulosin in LUT tissues may in part contribute to its clinically observed uroselectivity. A similar phenomenon has been reported for alfuzosin, a non-α₁ subtype-selective antagonist, whose functional uroselectivity in rats may be related to a higher concentration in the prostate than in plasma.26)

In conclusion, tamsulosin shows a sustained inhibitory effect on PE-induced IUP response in conscious male dogs. This prolongation is related to its higher concentration in prostate and urethra than in plasma and vascular tissues.

REFERENCES


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