Selective Binding of Tamsulosin to Genetic Variants of Human α₁-Acid Glycoprotein

Kazuhiko Hanada,* Naohiro Tochikura, and Hiroyasu Ogata

Department of Biopharmaceutics, Meiji Pharmaceutical University; 2–522–1 Noshio, Kiyose, Tokyo 204–8588, Japan.
Received April 26, 2007; accepted May 25, 2007; published online May 31, 2007

We investigated the characteristics of binding of tamsulosin to α₁-acid glycoprotein (AGP) genetic variants. The binding of tamsulosin to each of the human AGP variants was determined by ultrafiltration, and the binding characteristics for each variant were compared using binding parameters and inhibition of the binding by disopyramide and warfarin. The affinities of tamsulosin binding to an F1/S variant mixture and total AGP variants were relatively high (dissociation constants 1.6 μM). On the other hand, the dissociation constant for variant A was 14.9±2.53 μM. The binding of tamsulosin was competitively inhibited by warfarin but not by disopyramide. Tamsulosin appears to be a suitable compound for studying the characteristics of drug binding to human AGP F1/S variants under clinical conditions.

Key words tamsulosin; alpha-1 acid glycoprotein; genetic variants; human

Variations in protein binding affect the pharmacokinetics of drugs through changes in clearance and distribution volume. In general, for drugs that show a high degree of hepatic or renal extraction (flow-limited drugs) after intravenous administration, alterations of plasma protein binding cause changes in the unbound plasma drug concentration at steady state but little change in the total concentration. Recently, these theoretical changes have been confirmed clinically for propofol in vivo in terms of alterations in the pharmacokinetics.1) It is important to characterize the binding characteristics of such drugs in order to predict and understand alterations of drug disposition and also interaction between drugs. In particular, elucidation of drug binding site(s) on plasma protein is useful for predicting the pharmacokinetic alterations caused by drug–drug interaction.

Human α₁-acid glycoprotein (AGP) is separable isoelectrophoretically into several variants, known as the F1/S, and A variants.2–4) These variants are genetically determined and can be explained by the presence of two different AGP genes: the AGP-A gene encoding the variants ORM1 F1 and S, and the AGP-B/B’ gene encoding the variant ORM2 A.2,3) Herve et al. reported that disopyramide and imipramine, and warfarin, bind specifically to A variant and F1/S variant mixture, respectively, whereas chlorpromazine and lignocaine bind specifically to the F1/S variant to be a large hydrophobic area with no obvious structural requests for hydrophobic interactions.4–6) Accordingly, it has been suggested that human AGP has two main genetic variants for drug binding, and that each respective variant has different binding characteristics.

Three dimensional quantitative structure–activity model (3D-QSARs) analysis using CoMFA have been reported some common structural features and they showed A variant have two rings present in high affinity tricyclic drugs such as imipramine and high affinity of N-methylated amines.7) On the other hand, the binding site of F1/S variant to be a large hydrophobic area with no obvious structural requests for binding and they pointed out that the insufficient number of high affinity ligands for 3D-QSARs analysis.7)

Warfarin is a well known model compound for studies of binding to F1/S variants. However, warfarin is supplied as a racemate and its binding to AGP shows stereoselectivity.8) The affinity constant of S-warfarin for human AGP is higher than that of the R-isomer. Furthermore, warfarin shows wide inter-individual variation in its pharmacokinetics, and binds mainly to albumin. Therefore other suitable marker compounds are required for studying the characteristics of drug binding to the AGP F1/S variant in clinical situations.

Tamsulosin is an α₁-adrenoceptor antagonist used for the pharmacological treatment of benign prostatic hyperplasia, acting in part by relaxing prostatic smooth muscle (Fig. 1). Tamsulosin shows marked binding to AGP and that in human patients there is a significant correlation between the concentration ratio of bound and unbound (Cb/Cu) tamsulosin and the plasma AGP level, but not between Cb/Cu and the plasma albumin level.9) As the binding of tamsulosin was not significantly affected by the presence of amitriptyline, which binds specifically to the AGP A variant, tamsulosin may bind to the AGP F1/S variants with high specificity.

In the present study, we attempted to identify and quantify the characteristics of tamsulosin binding to human AGP variants.

MATERIALS AND METHODS

Chemicals and Reagents Tamsulosin hydrochloride and [³H]tamsulosin were gifts from Yamanouchi Pharmaceutical Co. (Tokyo, Japan). AGP (Lot No. 125H9329), disopyramide phosphate and warfarin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Immobiline dry plate and chelating Sepharose Fast Flow were obtained from Amersham-Pharmacelamb KLB (Uppsala). [³H]disopyramide was a gift from Nippon Roussel K.K. (Tokyo) and [¹⁴C]warfarin was obtained from Amersham Biosciences Limited (Piscataway, NJ, U.S.A.). Enantiomers of these drugs were separated enantioselective HPLC method and these stereochemical pu- rities were ascertained by stereoselective HPLC resolution reported previously.10) All other reagents used were of analyt-
ical grade.

Before application of radiolabeled compounds, purification by HPLC was performed. Once the purified radiolabeled drug had been dissolved in phosphate buffer, the solution was used within one experimental day. [1H]Tamsulosin was purified by adding 50 μl to 1 ml of saturated NaHCO₃ and then mixing it with 6 ml hexane:ethyl acetate (7:3, v/v). The mixture was shaken for 10 min, centrifuged, and then the supernatant was evaporated. Finally, the residue was reconstituted with phosphate buffer (pH 7.4).

Separation of AGP Genetic Variants The equilibration of immobilized copper(II) ions to iminodiacetate (IDA) Sepharose was performed at room temperature according to the methods reported previously.²⁻⁶,¹¹ Briefly, the IDA Sepharose gel loaded with copper(II) ions and equilibrated in buffer (20 mM sodium phosphate buffer, pH 7.0, containing 0.5 M NaCl) was packed into a glass column (1.5 cm I.D. x 30.0 cm L). A commercial AGP (10 mg/ml) was applied to the column at a flow rate of 10 ml/h. Fractions were collected and their respective absorbances were monitored at 280 nm. After the unbound variants (F1/S variant) had been eluted, the second elution buffer (buffer containing 20 mM imidazole) was applied to the column to remove the bound variant (A variant). The appropriate peak fractions were collected and concentrated on a YM 10 membrane filter. They were dialyzed against deionized water and lyophilized before use. The purities of the variants were checked by isoelectrophoresis as reported previously¹¹ and AGP variants greater than 98.5% of purity were used in this study.

In Vitro Protein Binding Study The binding of tamsulosin to human AGP was determined by ultrafiltration as described previously.¹¹ Briefly, 50 μl of tamsulosin (40, 60, 80, 100, 150, 200, 300 or 500 μM), 10 μl of [1H]tamsulosin and 10 μl of inhibitor at various concentrations (buffer as control, 0.5, 1.0 or 2.5 mM) were added to 450 μl of commercial AGP, purified F1/S or A variants and then the mixture was incubated at 37 °C for 15 min. A 450-μl aliquot of the mixture was poured onto a membrane filter (Ultrafree™-MC, MW cut-off 30000 for disopyramide).

Table 1. Parameters of Tamsulosin Binding to Human AGP (Total, F1/S Variant and A Variant)

<table>
<thead>
<tr>
<th></th>
<th>Total AGP</th>
<th>F1/S variant</th>
<th>A variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>0.67±0.09</td>
<td>0.91±0.15</td>
<td>0.97±0.07</td>
</tr>
<tr>
<td>Kd (μM)</td>
<td>1.57±0.46</td>
<td>1.31±0.29</td>
<td>14.9±2.53</td>
</tr>
<tr>
<td>α</td>
<td>0.27±0.08</td>
<td>0.09±0.02</td>
<td></td>
</tr>
</tbody>
</table>

n: number of binding sites; Kd: dissociation constant; α: non-specific binding constant. Each of the data represents mean±S.D.

Data Analysis We expressed non-specific binding (for very low-affinity binding sites) using constant α in the equation below.

\[
Ch = \frac{n \cdot AGP \cdot S}{Kd+S} + \alpha \cdot S
\]

Where \(Ch\), \(n\), \(AGP\), \(S\), \(Kd\) and \(α\) are the bound concentration of tamsulosin, number of binding sites, AGP concentration, unbound concentration of tamsulosin, dissociation constant of substrate, and non-specific binding constant for tamsulosin, respectively.

The drug binding parameters were determined by non-linear least squares regression analysis using WinNonlin (Pharsight Co., France). Selection of a preferable model was judged from both the apparent shape of the Scatchard plots and Aikaike’s information criteria (AIC) calculated by curve-fitting.

RESULTS AND DISCUSSION

The relationship between the unbound concentration and ratio of bound tamsulosin and AGP concentrations (r) in both the F1/S variant mixture and total AGP were comparable (Fig. 2). A two-binding-site model was used for analyzing the data for F1/S variants and total AGP, whereas a one-binding-site model was used for the A variant because the Scatchard plot was almost straight and the number of binding sites was lower than unity when the two-binding-site model was used. The \(Kd\) value of variant A was higher than that of the F1/S variant mixture (Table 1), indicating that tamsulosin bound strongly to the F1/S variant mixture. The ratio of the \(Kd\) of tamsulosin for the A and F1/S variants was 11.4 and that of warfarin was 7.3,⁵ indicating that the selectivity of these drugs for binding to the F1/S variant was comparable.
The number of binding sites for the F1/S variant mixture and the A variant was almost one, whereas that for total AGP was 0.67, which was reasonably explained by assuming that 70% of the total AGP used in this study consisted of the F1/S variants, and this was confirmed by isoelectric focusing (data not shown).

The binding of tamsulosin was competitively inhibited by S-warfarin (Fig. 3A), which is known to bind specifically to the F1/S variants, but was not significantly inhibited by S-disopyramide (data not shown). Furthermore, the binding of S-warfarin was also competitively inhibited by tamsulosin (Fig. 3B). These results indicated that tamsulosin binds specifically to the F1/S variants. Herve et al. reported that prazosin, which is also alpha-blocker, also showed selective binding to F1/S genetic variants of human AGP.7) The selectivity of tamsulosin for variant binding (estimated from ratio of dissociation constant) was 0.09, which was comparable for prazosin (0.17) and dipyridamole (0.06).7)

When considering the relationship between the unbound fraction of a drug and the AGP variants, one should take into account the specific binding to genetic variants and also the extent of non-specific binding to both variants, because the unbound fraction is determined by binding to both high- and low-affinity binding sites. The extents of nonspecific binding of tamsulosin and warfarin to the F1/S variants were also comparable (0.09 vs. 0.11), suggesting that tamsulosin and warfarin have similar characteristics of binding to these variants. Warfarin is available as a racemate and shows stereoselective binding to AGP, suggesting that only a mixed affinity constant for warfarin enantiomers is obtained if racemic warfarin is used for a binding study.

Warfarin binds mainly to albumin, shows large interindividual variation of its pharmacokinetics, and has serious side effects such as hemorrhage, making it unsuitable for studies of drug–drug interaction in clinical situations. On the other hand, tamsulosin shows no structural stereoisomerism, binds extensively to human AGP, and its plasma protein binding is changed in patients with renal failure or acute inflammation.12) It has been reported that there is a good correlation between the binding ratio (Cb/Cu) of tamsulosin and serum AGP concentration in humans, but not between Cb/Cu and albumin, indicating that the plasma protein binding of tamsulosin is sensitive to changes in AGP concentration in clinical situations.

In summary, we have demonstrated that tamsulosin binds to a F1/S variant AGP mixture with high specificity and would be a suitable compound for studying the characteristics of drug binding to AGP F1/S variants both in vitro and in vivo.

REFERENCES