Interaction of Propafenone Enantiomers With Human α_1-Acid Glycoprotein

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ABSTRACT   The interaction of propafenone enantiomers with human α_1-acid glycoprotein was studied using high-performance liquid chromatography. Each of the two optical antipodes interacted with one class of high-affinity binding sites characterized by $K_{a(R)}$ = (6.18 ± 0.93) × 10^5 M^{-1}, $n_{(R)}$ = 1.34 ± 0.09 for the (R)-isomer and $K_{a(S)}$ = (8.93 ± 1.82) × 10^5 M^{-1}, $n_{(S)}$ = 0.99 ± 0.08 for the (S)-isomer. Nonspecific binding to secondary low-affinity high-capacity binding site(s) was only slightly greater in the case of the (S)-enantiomer ($n'k'_{(S)}$ = (1.06 ± 0.09) × $10^4 M^{-1}$) compared to the (R)-enantiomer ($n'k'_{(R)}$ = (6.87 ± 0.72) × $10^3 M^{-1}$). It was concluded that both enantiomers interact with common single class of high-affinity binding sites on AAG (along with nonspecific binding) exhibiting only slight stereoselectivity for propafenone.

KEY WORDS: propafenone, enantiomers, α_1-acid glycoprotein

INTRODUCTION

Pharmacokinetic and pharmacodynamic differences involving stereoselective drug metabolism and stereoselective drug interactions have provided an important aspect in the clinical evaluation of some chiral compounds that are routinely administered in their racemic form.[1,2] For propafenone, a new class Ic antiarrhythmic agent (Fig. 1), the affinity of (S)-enantiomer for the β_2-adrenoceptor on human lymphocytes was demonstrated to be about 100 times greater than that of (R)-enantiomer.[3] On the other hand, the depression of the fast inward sodium channel was not sensitive to differences between propafenone enantiomers, suggesting that the chiral center of the molecule was not involved in this interaction. Differences in the pharmacokinetics of the enantiomers were recorded in patients with extensive metabolizer (EM) phenotype (established using sparteine). After administration of racemic propafenone, the (R)-enantiomer was cleared faster than the (S)-enantiomer leading to higher concentrations of the (S)-enantiomer in plasma. The patients with poor metabolizer phenotype (PM) were characterized by a loss of stereoselectivity. Contrarily, Brode et al.[4] reported in EM subjects preferential clearance of the (S)-enantiomer after separate administration of (R)- and (S)-propafenone. Besides stereoselective metabolic processes, differential protein binding may be responsible for the observed pharmacokinetic differences between enantiomers. Moreover, recent reports have demonstrated that the plasma protein binding of some antiarrhythmics[5–8] is stereocontrolled predominantly due to α_1-acid glycoprotein (AAG), thus contributing to their stereoselective pharmacokinetics.

The aim of this work was to study the binding of individual propafenone enantiomers to human α_1-acid glycoprotein by determining individual binding parameters of each enantiomer using high-performance liquid chromatography.

MATERIALS AND METHODS
Material and Chemicals

Human α_1-acid glycoprotein (G 9885) was from Sigma and was used without further purification. The (R)- and (S)-isomers with optical purity of 98.4 and 99%, respectively, were synthesised from (R,S)-propafenone · HCl (Knoll AG, F.R.G.) according to Lindner.[9] The buffer components KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$ were of analytical grade.

High-Performance Liquid Chromatography

The HPLC experiments were performed with a high pressure pump (HPP 5001, Laboratorni pristroje, Prague, Czechoslovakia), and eight-port switching valve equipped with 25 and 100 µl loops (Model PK 1, Vyvojove dilny, Czechoslovak Academy of Sciences, Prague, Czechoslovakia), a "compact glass cartridge" column (150 × 3.3 mm i.d.) packed with Lichrosorb Diol (Merck, Darmstadt, F.R.G., mean particle size 5 µm) and a variable-wavelength detector (LC Spectrophotometer Waters Lambda-Max Model 481, Bedford,
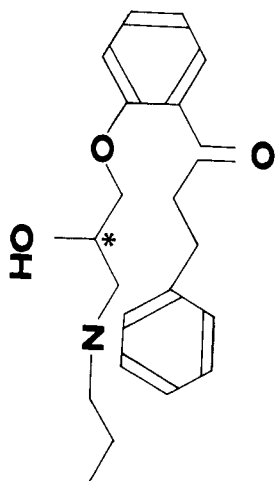
Fig. 1. Structural formula of propafenone (asterisk indicates the position of chiral center).

U.S.A.). Binding experiments were carried out at 37°C. The mobile phases were made from aqueous solutions of 0.067 M KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$ (pH 7.4) with addition of (R)- or (S)-propafenone · HCl in the concentration range 5×10^{-7}–$5 \times 10^{-4} M$. For dilute solutions detection was at 251 nm ($<1 \times 10^{-4} M$), or at 274 nm when drug concentrations were high (5×10^{-4}–$1 \times 10^{-4} M$). The flow rate of the eluents, which were degassed by helium, was in the range 0.5–1.3 ml/min. The samples injected were solutions of human AAG (10 μM, 0.44 g/liter) in phosphate buffer containing various amounts of (R)- or (S)-propafenone · HCl.

Binding was measured by using a drug solution saturating a nonchiral high-performance size exclusion column according to the method of Hummel and Dreyer[10] and described previously.[11,12] Injection of a protein sample into the eluent on to the column (pre-equilibrated with a fixed concentration of the ligand) led to a protein–ligand complex peak at the retention time of the protein followed by a negative peak at the retention time of the ligand. The bound quantity can be measured from the height of the above mentioned negative peak by internal calibration[11] (Fig. 2): the same quantity of protein is injected together with increasing amounts of ligand. The size of the negative peak decreases progressively and eventually it becomes positive, i.e., it varies linearly with the excess of injected ligand over the quantity in the same volume of eluent. By plotting the height of ligand peak versus the molar concentration of the drug in the samples, from the intersection with the x axis the exact quantity bound to the protein was calculated.

The advantage of the method of Hummel and Dreyer is that the protein–ligand complex does not dissociate during chromatographic analysis, even if the affinity constant is low, since the complex is always in equilibrium with the free ligand. Another advantage is the possibility of studying the interaction using a nonlabeled ligand (important especially in case of enantio-
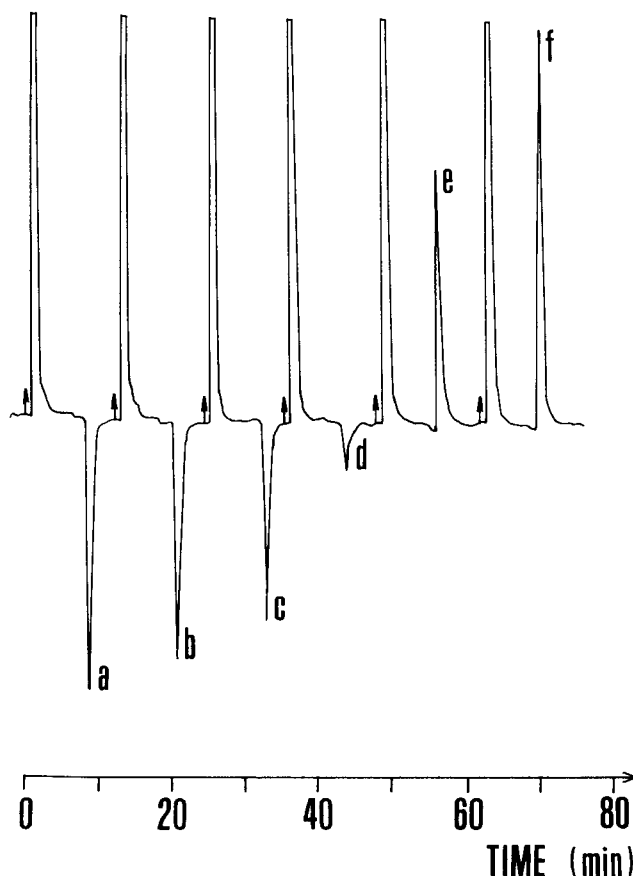

Fig. 2. HPLC elution profiles obtained after application of 25 μl of human AAG (10 μM) containing (R)-propafenone · HCl of 6.25×10^{-5} M (a), $1 \times 10^{-4} M$ (b), $1.25 \times 10^{-4} M$ (c), $2.5 \times 10^{-4} M$ (d), 5×10^{-4} M (e), $6.25 \times 10^{-4} M$ (f), injected on to a column previously equilibrated with solution of (R)-propafenone · HCl ($2.5 \times 10^{-4} M$) in phosphate buffer (0.067 M, pH 7.4).

mers). There are a few drawbacks of the method: the measurements are time consuming and the method requires a relatively large amount of ligand. Artifacts can occur when the ionic strength or the pH of the injected mixture is different from that of the eluent.

Evaluation of Binding Data

Scatchard analysis[13] of the binding data for both enantiomers (Fig. 3) revealed that each optical antipode interacts with two kinds of classes of binding sites present on AAG: one with high affinity and a small binding capacity and the other with low affinity and high binding capacity (termed nonspecific binding[14]).

The data were investigated also by the affinity spectra method.[15] The calculations started from the model with, for example, 100 classes of specific binding sites and one class of nonspecific binding and with one term for irreversible binding (Eq. 1):

$$B = \sum_{j=1}^{100} \frac{n_{(K_{Dj})}F}{F + K_{Dj}} + K_{nonsp}F + K_{ir} \qquad (1)$$

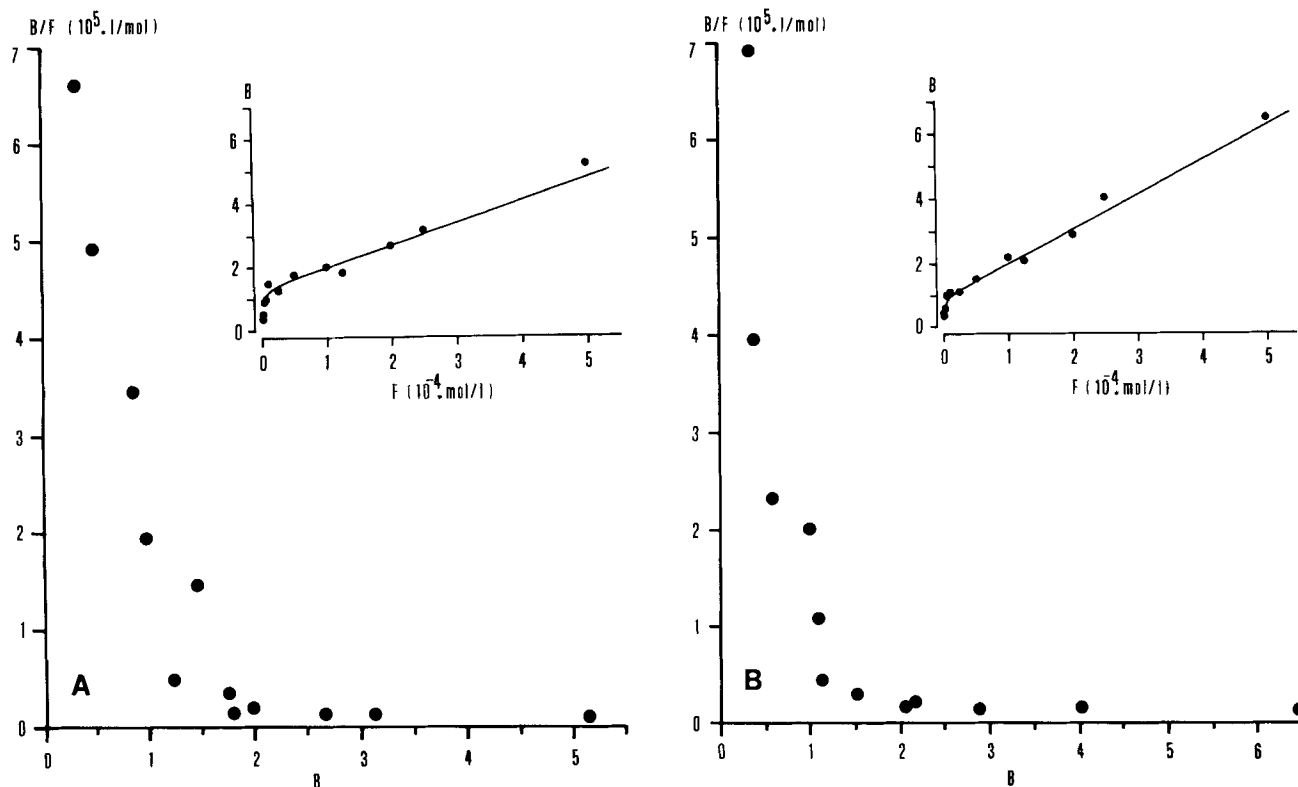where K_{Dj} is the succession of dissociation constant val-

Fig. 3. Interaction of (R)-propafenone (A) and (S)-propafenone (B) with human AAG at 37°C in phosphate buffer (0.067 M, pH 7.4) as a Scatchard plot (B/F vs. B) and as direct plot of bound (B) versus free (F) drug concentration (insets).

ues with sufficient density throughout the interval of interest (i.e., 5×10^{-7}–5×10^{-4} M); $n_{(K_{Dj})}$ is the number of binding sites per molecule of protein for the specific binding class with K_{Dj}; K_{nonsp} and K_{ir} represent the nonspecific and irreversible binding component, respectively; B is the concentration of bound drug bound by a mole of protein; and F is the free drug concentration. The result is that for only some K_{Dj} values is $n_{(K_{Dj})}$ significantly greater than zero. After statistical evaluations the program produces graphical output with a series of bell-shaped curves. From their number, location, and from their width one obtains the number of binding classes, their K_D, and the error of these K_D.

For both enantiomers there was one primary binding class. The nonspecific binding constant was calculated to be significant for both (R)- and (S)-enantiomers and the irreversible binding was in case of each enantiomer practically negligable.

Finally the data were investigated by extended nonlinear regression.[16] From the suggestions of previous two methods the following model was chosen:

$$B = \frac{nK_aF}{1 + K_aF} + n'k'F \qquad (2)$$

where K_a is the association constant of interaction concentration and the term $n'k'F$ represents the nonspecific binding component. The weighting scheme $1/F^2$ was used.

RESULTS AND DISCUSSION

Figure 3 shows the experimental data as a Scatchard plot and also a direct plot of bound versus free concentration for individual propafenone enantiomers. The binding of both optical isomers was concentration dependent, and the binding percentages varied within 9.4–86.8 and 11.5–87.5 for (R)- and (S)-propafenone, respectively, The highest drug concentration was limited practically by the solubility of (R)- and (S)-propafenone · HCl in water and there was no saturation of AAG binding site(s). Accordingly, Gillis et al.[17] were unable to characterize completely the binding parameters of racemic propafenone as they failed to saturate AAG. They reported that (R,S)-propafenone was strongly bound to AAG including two classes of binding sites both with high affinity ($K_{a1} = 1.31 \times 10^5 M^{-1}$, $K_{a2} = 1.67 \times 10^7 M^{-1}$) and low capacity ($n_1 = 0.79$, $n_2 = 0.20$). However, the model chosen by Gillis et al.[17] does not take into account the presence of chiral center in propafenone molecule (i.e., the diastereomeric nature of interaction of propafenone racemate, composed from 2 enantiomers, with AAG). Therefore the binding characteristics (2 classes of high affinity, low capacity binding sites on AAG) they present cannot be accepted as indicative of the true situation.

Our measurements of (R)- and (S)-propafenone binding characteristics revealed that each optical antipode interacted only with one class of high affinity binding

sites characterized by $K_{a(R)}$ = (6.18 ± 0.93) × $10^5 M^{-1}$, $n_{(R)}$ = 1.34 ± 0.09 for (R)-isomer and $K_{a(S)}$ = (8.93 ± 1.82) × $10^5 M^{-1}$, $n_{(S)}$ = 0.99 ± 0.08 for (S)-isomer. The nonspecific binding to secondary low-affinity, high-capacity binding site(s) was slightly higher in the case of (S)-enantiomer [(1.06 ± 0.09) × $10^4 M^{-1}$] compared to the (R)-enantiomer [(6.87 ± 0.72) × $10^3 M^{-1}$]. The most probable and molecularly plausible binding mechanism involved is the interaction of individual propafenone enantiomers with the common single class of high-affinity binding sites on AAG.[18] The limited number of available data in the literature[4] demonstrated relatively close degrees of plasma protein binding of propafenone enantiomers in man ($f_{u(R)}$ = 0.076 ± 0.015; $f_{u(S)}$ = 0.049 ± 0.012). Our results are in good agreement with these data suggesting that both propafenone enantiomers interact with AAG in a similar manner and that this binding exhibits practically no stereoselectivity at the therapeutic as well as at supra-therapeutic propafenone concentrations. Similarly the highly plasma protein bound antiarrhythmic drug asocainol (bound in human plasma up to 96% without apparent stereoselectivity[19]) binds to the major basic drug transport protein AAG with only borderline stereoselectivity.[18] In general the single drug binding site on AAG is reported to be only slightly stereoselective with stereoselectivity factors usually around 2.[18]

Although the stereoselective binding differences of propafenone do not appear large enough to be of clinical significance, it is of interest that the enantioselectivity of plasma binding increases with greater total binding, as described for propranolol.[5] This may be the case in subjects with higher plasma binding secondary to increased levels of AAG, i.e., in patients with inflammatory diseases[20] and acute myocardial infarction.[21] Yet the question concerning possible enantiomer–enantiomer interactions on AAG primary binding sites could not be answered so far. For example, Gross et al.[8] reported that the interaction of individual enantiomers of verapamil with AAG is the same when studied either separately or as pseudoracemate and consequently the binding of each individual enantiomer is not affected by the presence of the other enantiomer. Our binding experiments with propafenone racemate suggested that a more complicated mechanism may take part in the interaction of (R,S)-propafenone with AAG.[22] The competitive inhibition of the binding of propafenone enantiomers at a single site on AAG seems to be the most probable mechanism involved, such as described for other proteins by Jones et al.[23] and Knadler et al.[24] for 2-phenylpropionic acid and flurbiprofen enantiomers, respectively. Unfortunately, at present, an adequate method is not available to study the binding of propafenone enantiomers to AAG when present together in the same sample (as a pseudoracemic mixture).

Although our results have contributed to the understanding of the interaction of propafenone enantiomers with AAG, much remains to be learned about the pharmacokinetic consequences. The contradictory data available in literature claimed on the one hand substantially lower oral clearance of (R)-propafenone in the group of EM subjects after p.o. administration of individual enantiomers [50 ± 19% of the value for (S)-propafenone, i.e., enantiomeric ratio R/S = 0.50][4]; on the other hand, Kroemer et al.[3] described higher oral clearance of (R)-propafenone during long term oral therapy with racemic propafenone · HCl in the group of extensive metabolizers (R/S = 1.73).

In light of the slight protein binding differences observed between (R)- and (S)-enantiomers, it is unlikely that the dramatic differences in their pharmacokinetic behaviour could be attributed to differences in interaction with AAG. Further experimental studies with propafenone enantiomers are needed to get better insight into the cascade of stereoselective processes involving multiple enzymes, transport proteins, and receptors.

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