J. Pharm. Pharmacol. 1997, 49: 270–272
Received August 28, 1996
Accepted October 22, 1996

In-vitro Binding of Propiverine Hydrochloride and Some of its Metabolites to Serum Albumin in Man

P. MEISEL, STEFFI LANGNER AND W. SIEGMUND

Department of Pharmacology, University of Greifswald, 17487 Greifswald, Germany

Abstract

The distribution and pharmacological action of propiverine, a bladder spasmolytic agent, are affected by the extent of plasma-protein binding. Because attempts to assess the albumin-binding of propiverine have produced conflicting results, the binding parameters of the drug and some of its metabolites to serum albumin in man have been re-evaluated.

In man propiverine is bound to serum albumin at a single site with high affinity ($K_{A1} = 1{\cdot}45 \times 10^4$ L mol^{-1}) and at least two sites with low affinity ($K_{A2} = 2{\cdot}5 \times 10^2$ L mol^{-1}). The metabolites of propiverine, namely M2 (dealkylated propiverine), M5 (the *N*-oxide of propiverine) and M6 (the *N*-oxide of M2), are less firmly bound to serum albumin; this is considered to be non-specific binding. Binding experiments with human serum revealed that there are additional binding proteins. At therapeutic plasma levels the extent of binding was calculated to be 90, 15, 60, and 20% for propiverine and the metabolites M2, M5, and M6, respectively.

The strong binding of propiverine to serum proteins controls its availability to the liver. Because the metabolites are not tightly bound to serum proteins, after metabolism of propiverine its metabolites are easily eliminated.

Propiverine, a benzylic acid ester with a piperidine group, is a smooth-muscle relaxing agent effective by anticholinergic and myotropic mechanisms (Riotte & Mutschler 1987; Nomura et al 1989; Kaneko et al 1990). In man it is effective for treatment of bladder diseases with hyperactive detrusor vesicae and urge incontinence. Studies of the metabolism and the kinetics of propiverine in man have shown that it undergoes nearly complete degradation forming at least 13 metabolites (Müller et al 1993 and references cited therein). Because the distribution and pharmacological actions of a drug are affected by the extent of plasma protein binding, some attempts have been made to assess the albumin binding of propiverine (Mohr et al 1976; Tsuda et al 1991). The binding parameters published in these studies differ considerably–binding constants between 3×10^3 and 5×10^5 L mol^{-1} have been reported. The results are, moreover, prone to ambiguity as it was not shown whether or not propiverine was bound to saturable binding sites.

In this study, therefore, the binding parameters of propiverine and some of its metabolites to serum albumin in man have been re-evaluated in-vitro.

Materials and Methods

Materials

Crystalline human serum albumin (HSA) was purchased from Sigma, propiverine hydrochloride (α,α-diphenyl-*n*-propoxy-acetic acid 4-(1-methylpiperidyl) ester (Mictonorm)) and its metabolites M2 (dealkylated propiverine), M5 (the *N*-oxide of propiverine) and M6 (the *N*-oxide of M2) were provided by Apogepha Arzneimittel, Dresden, Germany. The structures of the compounds are shown in Fig. 1. HSA was dissolved in phosphate buffer (M/15, pH 7·4) to a final concentration of 400 μM and increasing amounts of the drugs were added. Molar ligand/protein ratios were varied between 0 and 1·5. Human serum was obtained by venipuncture from healthy volunteers and centrifugation of the clot.

Experimental

An ultrafiltration method employing Ultrafree-MC Filter Units (Millipore) with cut-off at 5000 Da was used to separate free and bound fractions. The test solutions were centrifuged for 10 min at 37°C and 2000 rev min^{-1}. Albumin-free drug solutions were handled in the same way to correct for non-specific binding to the tubes and filters. Free-drug concentrations were determined in the filtrate by two independent methods in order to circumvent the known uncertainties in the quantitative determination of propiverine. For assay the colorimetric method of Langley et al (1963) was modified. Briefly, the drugs were complexed with methyl orange, extracted into a mixture of 1,2-dichloroethane and isoamyl alcohol (19:1, v/v)

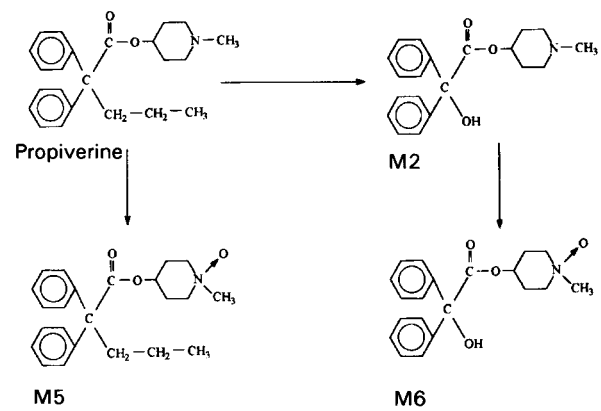

FIG. 1. The structures of propiverine and its metabolites M2, M5 and M6.

Correspondence: P Meisel, Department of Pharmacology, Ernst Moritz Arndt University Greifswald, Loeffler-Str. 23d, D-17487 Greifswald, Germany.

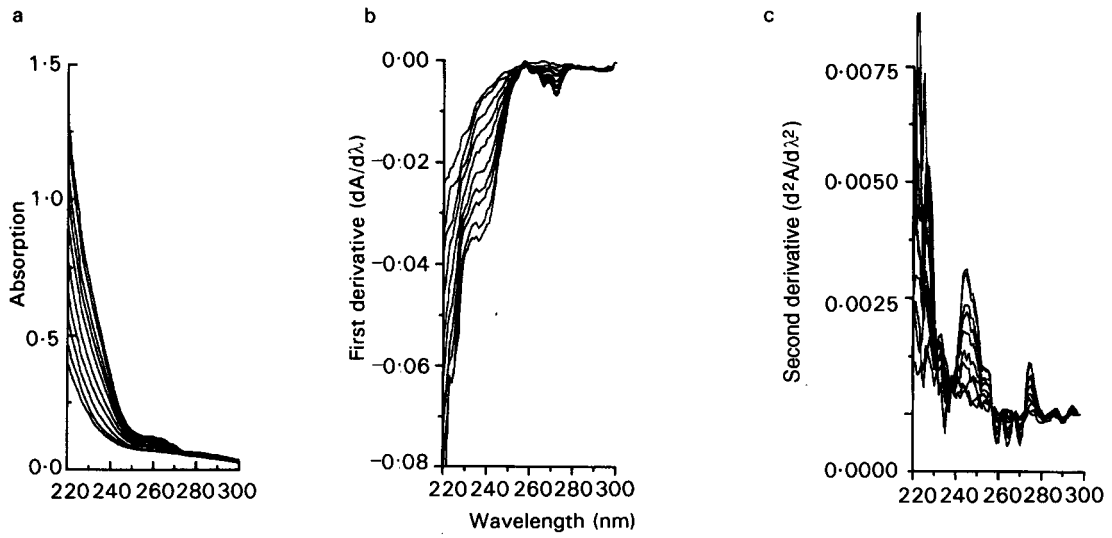

FIG. 2. Original traces, a, and first, b, and second derivatives, c, of propiverine hydrochloride spectra. Concentration range from 10–100 μmol L^{-1}, corresponding to 4–40 μg mL^{-1}.

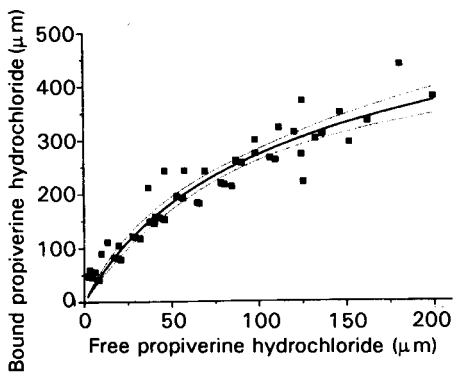

FIG. 3. Binding of propiverine hydrochloride to human serum albumin (400 μM final concentration). The solid curve shown is the best non-linear least-squares fit to the experimental points; the dotted line is the 95% confidence limit.

and after acidification with ethanolic sulphuric acid the absorbance was measured at 525 nm. To verify the results and to check if the membranes were impermeable to proteins a spectrum was recorded in the range 220–300 nm (Beckman diode-array SP2000 spectrophotometer) in which propiverine and its metabolites have no absorption maximum. The first and second derivative spectra were recorded and showed maxima accessible to quantification. The differences between $dA/d\lambda$ at the trough at 236 nm and a maximum at 255 nm were used to quantify the concentration of free drug (Fig. 2). Quantitative determinations of propiverine by use of the second derivative spectrum gave rather inconsistent results.

In binding experiments human serum was treated in the same way as the albumin solutions except for previous measurement of the albumin concentration in each sample by a dye-binding method with bromocresol green (Doumas et al 1971). Calibration curves were established by measuring standard concentrations of solutions of crystalline human serum albumin.

Data analysis

For calculation of the binding parameters the concentrations of free and total ligands were fitted by non-linear regression analysis (using the total concentration as independent variable and the ratio bound/total as dependent variable). The data were calculated by use of the equation:

$$B = (n_1[HSA]K_{A1}F)/(1 + K_{A1}F) + (n_2[HSA]K_{A2}F)/(1 + K_{A2}F)$$

where B and F are the concentrations of bound and free drug, respectively. The results are expressed as mean ± s.d.; fitted binding curves are shown with 95% confidence limits.

Results and Discussion

The plot B against F shows a saturable binding isotherm for binding of propiverine hydrochloride to albumin measured at 37°C (Fig. 3). The least-squares fitting procedure for this plot revealed that the drug was probably bound to a single site with a binding constant $K_{A1} = (1{\cdot}45 \pm 0{\cdot}14) \times 10^4$ L mol^{-1} and to at least two secondary sites of low affinity with binding constant $K_{A2} = (2{\cdot}53 \pm 0{\cdot}75) \times 10^2$ L mol^{-1}. In this low affinity range it is not possible to distinguish non-specific from low affinity binding unambiguously. In this case the free drug concentration increases linearly with increasing total drug concentration and albumin acts as a non-saturable carrier (Brodersen et al 1988). Recalculation of the data without limiting the number of binding sites to integers results in a one-site model with $n_1 = 1{\cdot}35 \pm 0{\cdot}15$ and $K_A = (1{\cdot}00 \pm 0{\cdot}20) \times 10^4$ L mol^{-1}.

In previous binding studies propiverine was used at therapeutic serum levels and, therefore, saturation of albumin was never approached. The molar drug/albumin ratio used was less than 0·1 in both studies mentioned. Reported figures for binding constants are $K_A = 3{\cdot}2 \times 10^3$ (Mohr et al 1976) and $K_A = 5 \times 10^5$ L mol^{-1} (Tsuda et al 1991). Re-plotting these data reveals that it is actually impossible to construct a rela-

Table 1. Parameters for binding of propiverine hydrochloride and its metabolites M2, M5, and M6 to human serum albumin.

Drug	Primary site binding constant K_{A1} (L mol^{-1})	Secondary site binding constant K_{A2} (L mol^{-1})	Percent bound in serum*
Propiverine	$(1{\cdot}45 \pm 0{\cdot}14) \times 10^4$	$(2{\cdot}53 \pm 0{\cdot}75) \times 10^2$	90–95
M2 (dealkylated propiverine)	-	$400 \pm 16^{\dagger}$	15
M5 (*N*-oxide of propiverine)	664 ± 37	-	60
M6 (*N*-oxide of M2)	-	555 ± 15	20

*At therapeutic plasma concentrations. †Increased proportion of bound M2 at molar ratios M2/HSA > 1.

tionship between the concentrations of free and bound drug from the experimental results. This might be because of the use of reciprocal transformations of the binding data (Scatchard plots) with arbitrary intercepts at the x-axis, a pitfall discussed by Klotz & Hunston (1979) some years ago. The value of the number of binding sites extracted from the binding experiments is, moreover, not necessarily an integer.

Human serum contains, in addition to albumin, other binding proteins. As far as the binding of propiverine is concerned, α_1-acid glycoprotein, in particular, might participate in keeping the free drug concentration low (Tsuda et al 1991). Using a pool of serum specimens from six volunteers we found that the amount of bound propiverine exceeded that expected from the albumin concentration. The albumin concentration in the test specimens was 580 μM (3·95 g dL^{-1}), the binding capacity was estimated to be (1460 ± 600) μM.

Compared with unmodified propiverine its metabolites have a significantly lower binding affinity to serum proteins, especially albumin. Because this type of binding obeys the equation $B = K_A \times F \times [HSA]$ it might even be considered as non-specific binding only. The binding parameters of all the compounds tested so far are shown in Table 1.

For all practical purposes comparisons should be made with therapeutic drug–plasma levels. After administration of 5 or 15 mg propiverine intravenously or orally, respectively, peak plasma concentrations in the range 0·1–0·5 μg mL^{-1} (0·25–1·25 μM) are obtained (Haustein & Hüller 1988). Using plots of bound/total against total drug concentration used in the binding experiments the percentage of the bound portion is calculated. Thus, although propiverine is highly bound to serum proteins, the metabolites are not (Table 1). As propiverine undergoes nearly complete decomposition by various hydrolytic and oxidative reactions (Hüller et al 1988) it might be expected that its distribution controls the availability to the liver where metabolism takes place. Conversely, protein binding affects the distribution characteristics of drugs. The pharmacologically active derivatives are generated by oxidation (M5 and M6). As those products are less firmly bound to proteins their pharmacokinetic fate is independent of binding for all practical considerations. In the event of intoxication, dialysis is probably possible with all the drugs tested, as proved by the ultrafiltration method used here to estimate protein binding. After metabolic transformation of propiverine, moreover, its metabolites are eliminated even more easily.

Acknowledgement

We wish to thank Mrs Ingrid Geissler for her skilful technical assistance.

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