A Simple Method for the Simulation of Unbound Serum Disopyramide Concentration in Patients

Satoshi KISHINO, Naonori KOHRI, Ken ISEKI, Katsumi MIYAZAKI,* Akikazu NOMURA, and Hisakazu YASUDA**

Department of Pharmacy* and Department of Cardiovascular Medicine,** Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan

(Received January 11, 1991)

There were remarkable differences in the serum total and unbound concentrations of disopyramide (DP) and mono-N-dealkyldisopyramide (MND), the major metabolite of DP, among patients with arrhythmias. Serum levels of α1-acid glycoprotein (AAG) also varied among the same patients.

To predict the unbound DP concentrations, we have obtained the serum concentration–time curves of unbound DP in these patients by means of total DP concentration, DP pharmacokinetic parameters, AAG levels and dissociation constants of DP and MND at specific AAG binding sites. In patients with normal AAG levels, the theoretical values of unbound DP were in good agreement with the measured concentrations. On the other hand, in patients with high AAG levels, the theoretical values could be obtained by correcting the calculated values using both AAG levels of each patient and the equation correlating the unbound DP fraction to AAG concentrations. These findings indicate that patient AAG levels can provide valuable information permitting the rapid estimation of unbound DP concentrations, the pharmacologically active fraction, and the development of effective DP dosage regimens.

Keywords — α1-acid glycoprotein; disopyramide; protein binding; simulation

Introduction

Disopyramide (DP) is an antiarrhythmic agent widely used in the treatment of supraventricular and ventricular arrhythmias.1–3) DP and its major metabolite, mono-N-dealkyl-disopyramide (MND) are bound to α1-acid glycoprotein (AAG) exclusively.4–6) Remarkable inter- and intraindividual variabilities in plasma protein binding in patients are associated with differences in AAG levels.4–10) Despite reports indicating that the pharmacological effect of DP correlates better with the unbound fraction of DP than with its total concentration,11,12) there is little information on inter- or intraindividual variability in serum unbound DP and MND concentrations in patients with arrhythmias. Routine determination of unbound DP concentrations is still impractical as with many other drugs due to the low concentration of unbound DP and technical analytical problems.13) Moreover, MND possesses weak antiarrhythmic activity, and a more potent anticholinergic effect.6,14) It is desirable, therefore, to develop a theoretical method which can predict unbound DP serum concentrations. In the present study, we measured DP, MND and AAG concentrations in patients with arrhythmias, and obtained a theoretical serum concentration–time curve of unbound DP using total DP concentrations, DP pharmacokinetic parameters, AAG levels and the dissociation constants of DP and MND at the binding site of AAG.

Materials and Methods

Chemicals and Blood Samples — DP (Nippon Roussel K.K., Tokyo, Japan) was kindly donated. Human AAG (Lot. 57F-9319) and human serum albumin (Lot. 117F-9311) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All other reagents were of the highest grade available commercially and were used without further purification.

The blood samples used in this study were obtained from seven healthy volunteers, four drug-free patients with mitral stenosis and five patients with arrhythmias treated with only DP for more
than 2 weeks on maintenance therapy. The patients had no history of renal and hepatic disease. Blood samples were obtained with plastic syringes at 0, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6 and 7 h after DP administration and were immediately transferred into glass tubes. Serum was separated by centrifugation at 1000 × g for 30 min after standing for 60 min. Serum samples were refrigerated at −20 °C until analysis. We ascertained that there were no differences in DP or MND or AAG concentration before and after sample storage.

**Protein Binding** — The protein binding of DP was determined by ultrafiltration using an ultrafree C3 LGC (Millipore ultrafiltration membrane, nominal molecular weight limit: 10000, Millipore Co., Bedford, MA, U.S.A.). DP and MND binding to ultrafilter membranes was negligible.

Binding studies for calculation of binding parameters were carried out using the serum obtained from healthy volunteers at 37 °C. Spiked serum samples were prepared by mixing serum and a known quantity of drug. DP and MND serum concentrations ranged from 1.0 to 50.0 μg/ml. The effect of MND concentration on DP binding was investigated at DP to MND concentration ratios ranging from 2.5 to 0.075.

Unbound fractions of DP were measured in spiked Sera (4.0 μg/ml of DP) with various AAG concentrations ranging from 67 to 187 mg/dl. The correlation between unbound DP fraction and albumin concentration was also examined at albumin concentrations ranging from 4.9 to 6.9 g/dl. A small volume of a known quantity of AAG or albumin saline solution, less than 4% of the serum volume, was added to minimize the effect of dilution of serum on the protein binding.

**Assay Procedures** — Serum and urinary concentrations of total DP and MND were determined by a minor modification of an established high performance liquid chromatographic (HPLC) method.15 Briefly, the procedure involved extraction of DP and MND into diethylether from alkaline serum or urine. The diethylether layer was evaporated to dryness in vacuo. The residue was reconstituted with 500 μl mobile phase (0.2 m ammonium acetate: methanol, 30: 70) containing the internal standard (Nicardipine), and 30 μl of the solution was injected into the HPLC system. Unbound serum drug fractions were determined by ultrafiltration. The ultrafiltration device was centrifuged in a fixed-angle roter at 5000 × g for 20 min. Ultrafiltrates were mixed with the internal standard and were injected into the HPLC system.

A liquid chromatograph (Hitachi 655A-11) equipped with a high pressure sampling valve (Rheodyne 7125) was used and a reverse-phase column (Hitachi 3053, 4.0 mm i.d. × 250 mm) was used for the stationary phase. The column was heated at 55 °C using a constant-temperature water bath circulator. The flow rate was 0.4 ml/min and pressure was approximately 55 kg/cm². Detection was at 270 nm using a variable wave-length ultraviolet (UV) monitor (Hitachi 638-41) at 0.005 a.u.f.s.

Serum AAG levels were measured by radial immunodiffusion (RID). Four-microliter aliquots of serum were incubated for 48 h in commercially prepared plates (Medical & Biological Laboratories Co., Nagoya, Japan). The diameter of the resultant precipitate was measured. Serum albumin concentrations were measured by the BCG method (A/G B-Test Wako, Wako Pure-Chemical Industry, Osaka, Japan).

**Pharmacokinetic Parameters** — Chart 1 shows the simplified model for analysis. Pha-
macokinetic analysis was conducted using the nonlinear least-squares computer program MULTI 16 following the model in Chart 1. The area under the plasma concentration–time curve (AUC) was calculated using the trapezoidal rule.

Estimation of Unbound Concentration — The protein binding of a drug follows the law of mass action and the relationship between bound and unbound drug concentrations can be described by the standard equilibrium equation:

\[ C_b = \sum_{i=1}^{m} \frac{n_i [Pt]}{K_{di}} \times C_f + C_f \]  

(1)

where \( n_i [Pt] \), \( K_{di} \), \( C_b \) and \( C_f \) are the total concentration, dissociation constant at the binding site, the bound and unbound drug concentration, respectively. In the presence of competitive inhibitor, MND, Eq. 1 can be arranged to Eq. 2:

\[ C_b = \sum_{i=1}^{m} \frac{n_i [Pt]}{K_{di} (1 + \frac{MND_i}{K_{mi}})} + C_f \]  

(2)

where \( K_{mi} \) and \( MND_i \) are the dissociation constant and the unbound concentration of MND, respectively.

Protein binding parameters were obtained from a scatchard plot using the MULTI program.16 The unbound drug concentration was calculated by means of Eqs. 2 and 3:

\[ C_t = C_f + C_b \]  

(3)

where \( C_t \) is the total concentration. The unbound (\( f_u \)) and bound (\( f_b \)) fractions were calculated as follows:

\[ f_u = 1 - f_b = \frac{C_f}{C_t} \]  

(4)

Statistical Analysis — A t-test was used to determine the statistical significance of differences.

Results

The AAG levels in cardiac patients and healthy subjects ranged from 63.0 to 104.0 mg/dl (mean ± S.D.: 87.6 ± 13.27 mg/dl, \( n = 9 \)) and from 41.0 to 76.0 mg/dl (mean ± S.D.: 61.25 ± 13.02 mg/dl, \( n = 7 \)), respectively. The mean AAG level was significantly \( (p < 0.05) \) greater in patients than in healthy subjects.

A typical serum concentration–time profile of DP and MND, and the pharmacokinetic parameters after oral DP administration to patients are shown in Fig. 1 and Table I, respectively. Serum concentrations and pharma-

Fig. 1. The Typical Serum Concentration–Time Profiles and Simulation Curve of DP (○) and MND (△) after Oral Administration of DP
(a) Patient S.Y., (b) H.Y., (c) S.W.
Table I. Pharmacokinetics Parameters in Five Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/d)</th>
<th>$AUC_{0-7}$ (h \cdot \mu g/ml)</th>
<th>$k_a$ (h$^{-1}$)</th>
<th>$k_e$ (h$^{-1}$)</th>
<th>$k_m$ (h$^{-1}$)</th>
<th>$k_n$ (h$^{-1}$)</th>
<th>$k_m$/$k_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.Y.</td>
<td>800</td>
<td>18.65</td>
<td>10.52</td>
<td>3.17</td>
<td>0.22</td>
<td>0.53</td>
<td>0.43</td>
</tr>
<tr>
<td>H.Y.</td>
<td>800</td>
<td>20.62</td>
<td>8.07</td>
<td>3.55</td>
<td>0.10</td>
<td>0.08</td>
<td>1.87</td>
</tr>
<tr>
<td>S.W.</td>
<td>450</td>
<td>18.62</td>
<td>1.36</td>
<td>0.52</td>
<td>0.14</td>
<td>0.03</td>
<td>0.20</td>
</tr>
<tr>
<td>I.W.</td>
<td>300</td>
<td>9.59</td>
<td>2.09</td>
<td>0.47</td>
<td>0.08</td>
<td>0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>K.H.</td>
<td>600</td>
<td>26.35</td>
<td>9.62</td>
<td>1.62</td>
<td>0.21</td>
<td>0.31</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Kinetic parameters were remarkably different among patients.

As shown in Fig. 2a, there was an obvious inverse relationship between the unbound fraction of DP and the AAG concentration. The coefficient of correlation was 0.9980. In contrast, the free fraction was not influenced extensively by presence of various albumin concentrations (Fig. 2b).

The Scatchard plots and Lineweaver-Burk plots for DP in the absence and presence of MND are shown in Fig. 3. Scatchard analysis indicates the presence of more than one binding site. The values obtained for the respective binding parameters of DP in the absence of MND were $K_{d1} = 5.83$ (μM), $K_{d2} = 202.92$ (μM), $n_1[Pt] = 16.25$ (μM), $n_2[Pt] = 67.92$ (μM). The Scatchard plot for MND in the presence of AAG was also obtained (data not shown). The MND protein binding parameters were $K_m1 = 1.06$ (μM), $K_m2 = 35.16$ (μM), $n_1[Pt]' = 5.41$ (μM), $n_2[Pt]' = 17.07$ (μM).

As shown in Fig. 3, the binding of DP to serum protein decreased with increasing concentrations of MND. Moreover, it was found that competitive inhibition between DP and MND occurred at AAG binding sites. These results suggest that only the first protein binding site would be inhibited. Therefore, the Eq. 5 defining the total serum concentration of DP ($C_t$) can be obtained from Eqs. 2 and 3.

$$C_t = \frac{n_1[Pt] \times C_f}{K_{d1} (1 + \frac{MND}{K_m1}) + C_f} + \frac{n_2[Pt] \times C_f}{K_{d1} + C_f} + C_f$$

(5)

And similarly, the relationship between total and unbound MND concentrations can be described by the following equation:

$$MND_t = \frac{n_1[Pt]' \times MND_f}{K_m1(1 + \frac{C_f}{K_{d1}}) + MND_f} + \frac{n_2[Pt]' \times MND_f}{K_m2 + MND_f} + MND_f$$

(6)

Fig. 2. The Relationship between Unbound Fraction of DP and (a) AAG or (b) Albumin Concentration

Concentration of DP: (a) 4.0 μg/ml, (b) 2.5 μg/ml.
Serum Unbound Disopyramide Concentration

As shown in Fig. 4a, the calculated value (solid line) was in good agreement with the measured unbound DP concentration in a patient, with the normal AAG levels (70 ± 16 mg/dl). The AAG level of this patient was 74 mg/dl. On the other hand, as shown in Fig. 4b, in a patient having a high AAG level (98 mg/dl) the calculated value (dashed line) was higher than the measured data. However, by correcting the theoretical value of $C_f$ using both this patient's AAG level and the correlation between unbound fraction and AAG concentration (Fig. 2a, $Y = -0.00215X + 0.4361$), the calculated value could be obtained. Another one case which fitted without correction of $C_f$ (AAG level: 78 mg/dl) and two cases which needed for the correction of $C_f$ (AAG levels: 98, 104 mg/dl) were obtained.

**Discussion**

The plasma or serum unbound drug concentration is a good index for the estimation of the pharmacological effect of an administered drug. Measurement of the unbound drug concentration is sometimes impractical due to the time-required for analysis and low unbound drug concentrations. Moreover, several basic drugs such as lidocaine, propranolol and quinidine are avidly bound to AAG like DP. Numerous physiological and pathological con-

---

**Fig. 3.** Scatchard Plots for DP in the Absence (○) and Presence (●) of MND

The inset is a Lineweaver-Burk plot of DP binding to serum in the presence of MND. Concentration of MND: 20.0 μg/ml.

where MND is the total MND concentration. In Eqs. 5 and 6, total serum concentrations of DP and MND were obtained using the equation for a one-compartment open model with a lag time in each patient. Finally the unbound DP concentration could be obtained by solving Eqs. 5 and 6 using the Newton Raphson method. Typical results are shown in Fig. 4.

**Fig. 4.** The Typical Serum Concentration-Time Profile of Total (○) and Unbound (●) DP in Patients

(a) The serum concentration-time profiles were obtained from a patient having a normal AAG level. The solid line is a curve simulated from the calculated value. (b) The serum concentration-time profiles were obtained from a patient having a high AAG level. The dashed and actual line are simulation curves before and after correction of calculated values, respectively.
ditions can alter AAG levels in serum, resulting in a change in the unbound fraction. There is little information for prediction of unbound concentrations of basic drugs such as DP in patients using biological indices such as AAG. In the present study, we have observed an increase of AAG levels and a decrease of unbound DP concentrations in patients with arrhythmias. These changes returned to normal levels with recovery from the disease (data not shown). Therefore, we developed a method to simulate the concentration–time profile of the unbound DP fraction. Theoretical values for unbound DP concentrations agreed well with the measured values when serum AAG concentration of patients were within the normal range (70 ± 16 mg/dl). On the other hand in patients with high AAG levels, good results could be obtained using the unbound (free) fraction calculated from the unbound drug fraction and AAG concentration. These results suggest that although large variations in serum AAG level may occur, the unbound DP concentration in each patient can be estimated provided DP and MND binding parameters have been obtained in advance. It is well known that DP used clinically is a racemic mixture of two enantiomers and that each has different pharmacokinetic properties. Therefore, the use of the present method to calculate the free fractions of DP enantiomers and several basic drugs should be investigated.

References

20) D. G. Shand: α1-Acid glycoprotein and plasma lidocaine binding, Clin. Pharmacokinet., 9, suppl. 1,