Effects of Repeated Clarithromycin Administration on the Pharmacokinetic Properties of Pindolol in Rats

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The goal of the present work was to determine the effect of clarithromycin (CAM) administration on the pharmacokinetic properties of pindolol in rats. The binding of pindolol to serum components increases proportionally with increasing α1-acid glycoprotein (AGP) concentration, indicating that AGP might play a major role in the binding of pindolol. After intravenous administration of pindolol to rats, the CAM-treated group showed a decrease in the volume of distribution, an increase in AUC and no change in the half-life as compared to the control group. Treatment with CAM increased the AGP concentration only. The serum concentration of albumin and creatinine, as well as the metabolic activity of hepatic microsomes towards pindolol, were not altered. Good correlation was observed between the AUC of pindolol in rats and the AGP concentration in serum. Moreover, at 5 min after the administration of an intravenous bolus dose of pindolol to CAM-treated rats, the free concentration of pindolol was lower but the total concentration was higher, compared with the control rats. These results suggested that the influence of CAM on the pharmacokinetic properties of pindolol in CAM-treated rats can be explained by protein binding which, in turn, may be associated with variations in AGP concentration.

Key words α1-acid glycoprotein; protein binding; clarithromycin; pindolol

Clarithromycin (CAM), a 14-membered macrolide antibiotic, is widely used in the treatment of infectious disease. In recent years, a number of studies have provided evidence suggesting that CAM may be effective in the treatment of chronic lower respiratory tract inflammation. If true, this could be due to its anti-inflammatory activity and not its antimicrobial action.1-3) These observations suggest that studies of clinical applications of CAM should be extended. It is also noteworthy that CAM is considered to be a relevant inhibitor of CYP3A, based upon its effects on pharmacokinetics of drugs such as cyclosporin and terfenadine.4,5) Thus, it is possible that drug interaction may occur when CAM is co-administered with drugs which are metabolized by CYP3A.

It is well known that α1-acid glycoprotein (AGP) is the major binding protein of cationic drugs, including propranolol, disopyramide and carbamazepine,6) and that this binding has been confirmed as an important determinant of interindividual variability in the binding of basic drugs.7) In addition, AGP concentrations in sera increase several-fold under conditions of stress, such as surgery, inflammatory diseases, cancer or myocardial infarction, because AGP is a major acute phase reactant.8,9) Increased AGP levels can also be observed after repeated administration of certain enzyme-inducing drugs, especially phenobarbital, phenytoin and rifampicin, although the mechanism for this induction is not clear.7,10) Such elevations in serum AGP may affect the serum protein binding of a variety of drugs that bind extensively to AGP.11,12) As a result, this alteration in plasma protein binding may influence the pharmacokinetic properties of drugs.

Recently, it has been reported that the CYP3A gene family is induced in some species by the AGP inducers, phenobarbital and rifampicin.13,14) However, besides the previous reports that CYP3A protein levels increased after CAM treatment,15) no comprehensive studies with regard to the effect of CAM on AGP have been carried out. In this study, in order to investigate potential drug–drug interactions in the co-administration of a drug with CAM, we employed the β-adrenoceptor blocking agent, pindolol, as a model drug, since it binds strongly to AGP.12,16) Pindolol is mainly metabolised by the liver with a residual of about 20% being excreted unchanged in the urine. The goal of the present work was to assess whether repeated administration of CAM affects the disposition of pindolol through CAM-induced changes in AGP concentration and subsequent influence on the serum protein binding of pindolol.

MATERIALS AND METHODS

Materials CAM was a generous gift from the Taisho Pharmaceutical Co., Ltd. (Saitama, Japan). Pindolol and the Creatinine-HA test Wako were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Antipyrine was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Rat serum albumin (RSA) and AGP were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were of reagent grade.

Animals and Treatment Male Wistar rats, weighing 200–250 g, were used and maintained in an air-conditioned room under controlled temperature (22–25 °C) and humidity (50–60%). They were fed with commercial rat chow and had free access to tap water. The animals were used for the experiments after 3 d of acclimation.

CAM was dissolved in 10% dimethyl sulfoxide (DMSO) and was administered at a dose of 5 mg/kg s.c. for 7 d. 24 h after the last injection of CAM or vehicle, pindolol was administered at a dose of 3 mg/kg i.v. Serum samples for pindolol analysis were taken at 5, 15, 30, and 60 min post-administration. Blood samples were centrifuged and the resulting serum was stored at −20 °C until further analysis.

HPLC Analysis for Pindolol Standard curves for pindolol assays were generated from the serum collected from untreated rats of the control group. Antipyrine was added to each 0.3 ml serum sample as an internal standard. Six milli-
liters of ether and 0.3 ml of 1 N NaOH were added, and the samples were then gently shaken for 10 min. After centrifugation at 1500×g for 5 min, the organic phase (upper layer) was evaporated to dryness. The samples were then reconstituted with 0.05 ml of 0.02 M KH₂PO₄/acetonitrile (7:3, v/v), the mobile phase for the HPLC system. The samples were analyzed by HPLC using ultraviolet detection at 264 nm. The mobile phase was pumped at 0.7 ml/min through a 7 μm RP-Select B reverse phase column.

**Preparation of Microsomes** Rats were weighed and then sacrificed by decapitation and the livers perfused with ice cold saline, rinsed and homogenized in 3 volumes of 0.154 M KCl and 0.01 M sodium potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10000×g for 20 min. The supernatant was then centrifuged at 100000×g for 60 min. Microsomal proteins were determined by the method of Lowry et al.¹³

**Enzyme Assay** *In vitro* incubations of pindolol with rat hepatic microsomes were performed at 37°C. The final assay volume was 0.5 ml of a solution composed of 0.1 M potassium phosphate buffer (pH 7.4), EDTA (0.1 mM), magnesium chloride (3 mM), microsome protein (1 mg/ml), and pindolol (2–20 μM). After a 5 min preincubation period at 37°C, the reactions were initiated by the addition of an NADPH-generating system containing β-NADP⁺ (1 mM), D-glucose 6-phosphate (5 mM), and D-glucose 6-phosphate dehydrogenase (1 unit/ml). Reactions were terminated by the addition of 0.5 ml of ice cold 0.1 M NaOH.

**Assays for AGP and RSA** AGP and RSA concentrations in rat serum were measured by single radial immunodiffusion.²⁰ 5 μl of serum samples were placed in holes in a 1% agarose gel containing antiserum and were allowed to stand for 48 h at room temperature to yield a fully developed precipitin ring.

**Data and Statistical Analysis** Pindolol serum concentration vs. time data were analyzed by means of a one-compartment model with nonlinear least-squares regression.²¹ The total area under the serum drug concentration versus time curve (AUC) was estimated by the linear trapezoidal rule. The half-life (t₁/₂) and volume of distribution (Vₘ) were calculated by the usual procedures.

Correlations were assessed by least-squares linear regression analysis. Statistical evaluations were performed on various parameters with a paired Student's t-test.

**RESULTS**

**Effects of Repeated CAM Administration on the Disposition of Pindolol** After intravenous administration to rats, the concentration of pindolol in CAM-treated rats was significantly higher than that in the control rats (Fig. 1). The pharmacokinetic parameters of pindolol are summarized in Table 1. Treatment with CAM caused a decrease in the volume of distribution and an increase in the AUC of pindolol, while the half-life remained unchanged, due to compensation from the decrease in total clearance and volume of distribution. In order to better understand the modulation of the pharmacokinetic properties of pindolol as the result of repeated CAM administration, the elimination of pindolol, including hepatic metabolism, was examined. However, CAM treatment had no effect on serum creatinine levels (0.27±0.01 to 0.28±0.01 mg/dl) nor on the apparent rate constant for the elimination for pindolol in rat liver microsomes (3.2±0.3 to 3.1±0.5 h⁻¹). We also examined the concentration of serum protein, since albumin and AGP represent important drug binding proteins in serum. No significant differences in serum albumin concentration were observed for the control (3.9±0.1 g/dl) vis-a-vis the CAM-treated rats (3.6±0.1 g/dl), but the AGP concentration in serum increased significantly from 19.7±0.9 to 48.7±4.1 mg/dl after multiple doses of CAM. These results lead us to speculate that the serum binding of pindolol might increase in CAM-treated rats.

**Serum Protein Binding** The binding of pindolol was greater in CAM-treated rat serum compared to the control serum. CAM treatment produced a nearly 2.5-fold increase in AGP concentration, and the binding of pindolol (6 μM) was increased from 34.5% to 66.5% (Table 2). An increase in the binding of pindolol was also observed in a protein binding experiment using AGP solutions ranging from 20 to 60 mg/dl. However, the addition of CAM to either serum or AGP solutions only negligibly affects the *in vitro* binding of pindolol, and the binding of pindolol by 4 g/dl albumin was

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**Fig. 1. Serum Concentration of Pindolol Following Intravenous Administration at a Dose of 3 mg/kg to Control or CAM-Treated Rats**

Open and closed circles represent control and CAM-treated groups, respectively. Each value and vertical bar represents the mean±S.E. (n=7). *p<0.05, when compared to control group.

<table>
<thead>
<tr>
<th>Table 1. Pharmacokinetic Parameters after Intravenous Administration of Pindolol to Control and CAM-Treated Rats</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
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<tr>
<td>Vₘ (l/kg)</td>
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<td>AUC (μg·h/ml)</td>
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Results are mean±S.E. of eight rats per group. *p<0.05; **p<0.01, when compared to control group.

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<th>Table 2. Binding of Pindolol to AGP, RSA and Serum in the Presence or Absence of CAM</th>
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<td>Percentage binding (%) of pindolol</td>
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<tr>
<td>Pin (6 μM)</td>
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<tr>
<td>AGP (20 mg/dl)</td>
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<td>AGP (60 mg/dl)</td>
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<tr>
<td>RSA (4 g/dl)</td>
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<tr>
<td>Control serum</td>
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<td>CAM-treated serum</td>
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constantly about 30%, with the pindolol concentration ranging from 2 to 18 μM. Therefore, it seems likely that AGP concentration determines the extent of pindolol binding in serum.

As shown in Fig. 2, a good correlation was observed between the AUC of pindolol and AGP concentration, but not with respect to the albumin concentration. In addition, it should be noted that a similar correlation exists between AGP concentration and the unbound fraction of pindolol in vitro.

At 5 min after an intravenous bolus administration of pindolol, significant differences in protein binding and the concentrations of pindolol between the control rats and CAM-treated rats were observed (Table 3). These in vivo results are consistent with the in vitro results. Furthermore, the concentrations of free drug in control rats and CAM-treated rats were 1.03 and 0.77 μg/ml, respectively. This difference can be attributed to differences in the serum binding of the drug.

DISCUSSION

It is generally accepted that only unbound drug is available for pharmacological action and for the processes of distribution and elimination. Treatment with CAM increased AGP concentration in serum without affecting the albumin and creatinine concentration, as well as the apparent elimination of pindolol in liver microsomes. In addition, repeated treatment with CAM increased AGP mRNA levels in rat liver in a dose-dependent fashion. This increase in AGP mRNA was detectable after 12 h of CAM treatment, and reached a maximum by 2 d. Since maximal serum protein levels were found at 3 d after the first injection of CAM, the increase in AGP mRNA caused by CAM might be expected to be followed by the corresponding changes in serum AGP. Thus, it appears that the increased serum binding of pindolol in CAM-treated rats was the result of a significant increase in AGP concentration. This change in protein binding could directly influence the pharmacokinetic properties of pindolol. Furthermore, interestingly, the free and total drug concentrations were lower and higher, respectively, in CAM-treated rats compared to those in control rats. In the case of the control group, a variation in drug binding elicited a change in both total and free concentration in serum. Based on an earlier study, which concluded that the pharmacological action of propranolol is better correlated with its free concentration, than with the total concentration in serum, it is possible that alterations in the pharmacodynamics of pindolol in CAM-treated rats may occur as a consequence of a change in free drug concentration.

In conclusion, the present findings suggested that the influence of CAM on the pharmacokinetic properties of pindolol in CAM-treated rats can be reflected in protein binding which, in turn, may be associated with variations in AGP concentration.

REFERENCES