Clinical Oral Doses of Dexamethasone Decreases Intrinsic Clearance of Quinidine, A Cytochrome P450 3A Substrate in Dogs

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ABSTRACT. We investigated the effect of dexamethasone (DEX) at clinical doses on the pharmacokinetics of quinidine (QN) in dogs. Dogs (5 healthy 1-year-old male beagles) were orally administered DEX once daily for 5 days at 2.5 or 7.5 mg/day. QN (2 mg/kg) was intravenously injected 3 weeks before and one day after the DEX treatment. The plasma concentration of QN was determined by high-performance liquid chromatography with fluorometric detection. Plasma concentrations of albumin and α1-acid glycoprotein (AGP) were determined by a bromocresol green method and a single immunodiffusion method, respectively. In order to calculate unbound concentrations of QN in plasma, the binding kinetics of QN in plasma was examined by an ultrafiltration method using pooled plasma from the 5 dogs when they were drug-free. Total body clearance of QN was decreased dose-dependently. By the DEX treatment, although the decrease was not statistically significant. Elimination half-lives significantly increased (more than twice at 7.5 mg), and intrinsic clearance significantly decreased (about 50%). The volume of distribution increased significantly (about two-fold). Plasma levels of AGP significantly decreased, and the unbound fraction of QN in plasma significantly increased. Our results demonstrate that clinical doses of DEX significantly affect the pharmacokinetics of QN, a CYP3A substrate in dogs, by decreasing CYP3A activity and plasma AGP levels. There is a possibility that adverse drug-drug interaction occurs during DEX therapy through its effects on CYP3A activity and plasma AGP levels.

KEY WORDS: canine, clearance, CYP3A, dexamethasone, quinidine.

Drug metabolism is one of the determinant factors of drug disposition. Alterations in drug metabolism are, therefore, a matter of primary concern for many researchers not only in human medicine, but also veterinary medicine. Although altered drug metabolism may result from several factors, enzyme inhibition has been extensively studied and recognized as the most important factor requiring a great deal of attention in the clinical state because it may lead to fetal toxicity of co-administered drugs in some cases [6].

DEX is a well-established inducer of the cytochrome P450 (CYP) 3A subfamily. There are many reports that describe induction of CYP3A through in vitro [14, 10] and in vivo studies [7, 17, 19]. However, very high doses (more than 10 times of the clinical dose) were used in most of the in vivo studies. In canine clinics, the recommended doses of DEX are 0.05–0.3 mg/kg/day for anti-inflammatory therapy and 0.5–1 mg/kg/day for acute adrenocortical collapse and immunosuppressive therapy [4]. In our previous study [21], therefore, we examined the inducing effect of DEX on CYP3A using clinical doses in dogs and rats. Although Jayyosi et al. reported that there is no significant induction of CYP3A in dogs treated with DEX (50 mg/kg for 5 days), we observed a decrease of about 70% in the maximum reaction velocities of midazolam 4-hydroxylation in hepatic microsomes from dogs and rats treated with DEX at 0.75 mg/kg/day for 5 days without alteration of the Michaelis-Menten constants. Although DEX inhibited the metabolic reaction in a competitive manner, its inhibitory constant was relatively large (about 100 µM). In addition the drug was not a mechanism-based inhibitor. We, therefore, concluded that the decrease in the maximum reaction velocities was derived from a down-regulating effect of DEX on CYP3A.

Comedication of other drugs with DEX is not infrequent in veterinary medicine. The down-regulating effect of DEX must affect the pharmacokinetics of comedicated drugs and lead to undesirable drug-drug interactions in some cases if the drugs are CYP3A substrates. Evaluation of the decrease in CYP3A activity in the intact condition, therefore, presents important information for veterinary clinicians. Because of this, we examined the effect of clinical doses of DEX on the pharmacokinetics of a CYP3A substrate, quinidine (QN), in dogs. We selected this drug because it is mainly metabolized by CYP3A [11] and is subjected to low hepatic extraction in dogs [2].

MATERIALS AND METHODS

Animals: Five beagles (male, 1-year-old, 11–15 kg) used for QN-pharmacokinetics studies were obtained from CSK Research Park (Nagano, Japan). The dogs had access to water ad libitum and were given food twice daily (8 AM and 8 PM).

Materials: DEX was purchased as a tablet from Asahi Kasei Corporation (Osaka, Japan). Quinidine sulfate (QN) was obtained from Nikken Chemicals Co., Ltd. (Tokyo, Japan). All other chemicals used as regents were of analyt-
**QN pharmacokinetic study**: All of the studies were performed in our laboratory. This experiment was conducted in accordance with the guidelines for the care and use of laboratory animals of the Faculty of Agriculture at the Tokyo University of Agriculture and Technology.

DEX tablets were administered orally once daily between meals (2:00 PM) for 5 days at a dose of 2.5 or 7.5 mg (0.17–0.23 or 0.50–0.68 mg/kg/day). A randomized crossover study design was used for DEX administration. QN was intravenously injected at 2 mg/kg 3 weeks before the start of the DEX treatment and one day after the final dosing of DEX. After 4 weeks, the animals were treated again with DEX at a dose of 2.5 or 7.5 mg for five days, and then QN was injected. The QN solution for intravenous administration was prepared by dissolving 250 mg of quinidine sulfate in 2 ml of ethanol and adjusting total volume to 50 ml using sterile pyrogen free water, yielding 5 mg/ml of QN solution. Blood samples (1.5 ml) were collected at 0.5, 1, 2, 3, 4, 6, and 9 hr after the QN injection in EDTA-containing test tubes. The blood samples were centrifuged at 2,000 × g for 5 min, and the obtained plasma was stored at −20°C until QN determination.

In order to examine the effect DEX treatment on plasma concentrations of albumin and α1-acid glycoprotein (AGP), plasma samples (1 ml) were collected before DEX treatment and just before the QN injection. The plasma samples were stored at −20°C until protein determination.

**QN protein binding kinetic study**: About 20 ml of blood was collected from the five dogs when they were drug-free. The obtained plasma was pooled, and then used for the study. The plasma protein binding of QN was examined by an ultrafiltration method using a Centrifree Micropartition System® (Grace Japan KK-Amicon, Tokyo, Japan). QN solution (50 µl) was added to 950 µl aliquots of plasma at various concentrations (2–500 µM). The mixture was incubated at 37°C for 10 min to allow drug-protein binding equilibrium, pipetted into the sample reservoir of the ultrafiltration device, and then centrifuged at 2,000 × g for 10 min. The concentration of QN in the filtrate was determined immediately after centrifugation.

**Determination of QN concentrations**: Plasma QN concentrations were determined using high-performance liquid chromatography (HPLC) with fluorometric detection. The mobile phase was a mixture of water containing 0.4% triethylamine (pH adjusted to 2.5 using phosphoric acid) and acetonitrile (90:10, v/v) that was pumped at a flow rate of 1 ml/min. An C8 column (LiChrospher 100 RP-8, 4.6 × 250 mm, Kanto Chemical Co., Inc., Tokyo, Japan) was used as an analytical column. Fluorescence was monitored at an excitation wavelength of 340 nm and emission wavelength of 425 nm. After acetonitrile (750 µl) was added to 250 µl of plasma, the mixture was centrifuged at 10,000 × g for 2 min, and 500 µl of the supernatant was evaporated to dryness under reduced pressure using a rotary evaporator (Rotavapor R-114, Shibata Scientific Technology, Ltd., Tokyo, Japan). The residue was reconstituted with 250 µl of the mobile phase, and 50 µl of the solution was injected into an HPLC column. The recovery of QN at relevant concentrations averaged 89.6 ± 1.7% (n=4). The inter-day CV values were 1.93 to 7.93% at 100 ng/ml (3 days, 4 determinations/d), with a limit of quantification of 10 ng/ml at a signal-to-noise ratio of 3.

Plasma concentrations of albumin were determined by a bromcresol green method [8] using a commercial kit (Albumin Test Wako, Wako Pure Chemical, Osaka, Japan). Plasma concentrations of α1-acid glycoprotein (AGP) were determined by a single radial immunodiffusion method [1], using a commercial kit (Canine α1-AGP Plate, Saikin Kagaku Research Institute, Sendai, Japan).

**Pharmacokinetic analysis**: The pharmacokinetics of QN after intravenous injection was analyzed using a one-compartment model. The initial concentration (C0) and elimination rate constant (kel) were calculated using the nonlinear least squares regression program “MULTI” described by Yamaoka et al. [20]. Pharmacokinetic parameters including elimination half-life (t1/2), total body clearance (CLint), and volume of distribution (Vd) were calculated using the conventional methods.

Scatchard plot analysis showed that plasma protein binding of QN consisted of specific high-affinity binding and non-specific low-affinity binding plot. Subsequently, the relationship between the bound concentration (C bind) and unbound concentration (Cf) in the plasma was shown using the following equation,

\[
C_b = \frac{B_{max} \times C_f}{K_d + C_f} + A \times C_{f} \quad \text{Equation 1}
\]

Where Bmax and Kd are the binding capacity and dissociation constant for the high affinity site, respectively. A is a proportionality constant for the low affinity site (possibly albumin and lipoprotein).

Since Ikenoue et al. [8] indicated that the high affinity binder for QN is AGP in dog plasma, the Cf of QN at each sampling point after intravenous injection was calculated using the total concentration (Ct) and AGP concentration, the binding kinetic parameters, and the following equations:

\[
C_b = \frac{B_{max} \times AGP_{cont} \times AGP_{DEX} \times C_f}{K_f + C_f} + A \times C_{f} \quad \text{Equation 2}
\]

\[
C_b = C_t - C_f \quad \text{Equation 3}
\]

\[
\text{AGP}_{cont} \text{ and AGP}_{DEX} \text{ are the plasma AGP concentrations before and after DEX treatment, respectively. From the } C_f \text{ at each sampling point, we calculated the AUC of } C_f \text{ (AUCf), and then the intrinsic clearance using the following equation:}
\]

\[
CL_{int} = \frac{\text{Dose}}{\text{AUCf}}
\]
Dexamethasone decreases quinidine clearance

Statistical analysis: Differences in the plasma concentrations and pharmacokinetic parameters of QN between absence and presence of DEX were analyzed by paired Student’s t-test and were regarded as statistically significant when p values were below 0.05.

RESULTS

Effect on QN pharmacokinetics: Figure 1A shows the plasma concentration-time curves of QN after intravenous injection at 2 mg/kg with or without oral DEX treatment. DEX treatment caused the slope of the curves to become flat. Nine hours after QN injection, the plasma QN concentrations were about 2-fold higher in dogs given a DEX treatment of 2.5 mg/day and about 4-fold higher in dogs given 7.5 mg/day compared with dogs that were not treated. In the early stages after QN injection, however, the plasma QN concentrations were significantly lower in dogs treated with DEX.

As shown in Table 1, DEX treatment tended to decrease in a dose-dependent manner, although the decrease was not statistically significant. DEX treatment increased elimination half-lives by more than 2-fold at 7.5 mg/day. There was a significant increase in as the DEX treatment, and this resulted in lower QN concentrations in the plasma in the early stages after QN injection.

Effect on plasma albumin and AGP: As seen in Table 2, the plasma AGP concentrations were decreased significantly by the DEX treatment. The values for AGP were approximately half of those before treatment. However, the plasma albumin concentrations were unchanged.

QN protein binding: From 6 hr after intravenous injection of QN at 2 mg/kg, the QN unbound concentrations in the plasma were lower than the quantification limit in dogs not treated with DEX. Thus, the binding kinetics of QN was also examined in an in vitro study to calculate the unbound concentrations in plasma after QN injection with or without DEX treatment. As shown in Fig. 2, the Scatchard plot showed two binding modes. For the specific high-affinity binder, the binding capacity (Bmax) was 8.25 µM and the dissociation constant (Kd) was 0.23 µM. For the non-specific low-affinity binder, the proportionality constant (A) was 0.80 (Fig. 2).

Using these parameters, the AGP concentrations, and total QN concentrations in the plasma, the unbound concentrations of QN in plasma were calculated using Equations 2.
and 3 (Fig. 1B), because Ikenoue et al. has indicated that AGP is a high-affinity binder in dog plasma [8]. In contrast to the total concentrations in Fig. 1A, the unbound concentrations at t=0 hr seemed to be similar among the 3 treatments. At later stages after QN injection, however, the differences in the unbound concentrations among the treatments were more evident compared with those in the total concentrations. The unbound fraction (fu) of QN increased from 0.0976 ± 0.0242 to 0.166 ± 0.027 (Table 2). CLint, calculated by dividing the dose by the AUC of the unbound drug (AUCf), was decreased by about half by the DEX treatment (Table 1).

**DISCUSSION**

Our previous study [21] demonstrated decreased activities in CYP3A and 2D using hepatic microsomes from dogs orally treated with DEX at clinical doses (0.17–0.23 mg/kg/day and 0.51–0.68 mg/kg/day, respectively) for 5 days. Michaelis-Menten kinetic analysis suggests that this decrease results from a down-regulating effect of DEX on these CYPs. In the present study, these doses of DEX significantly affected the pharmacokinetics of QN. After DEX treatment at 7.5 mg/day for 5 days, the half-life of QN significantly increased. The DEX treatment also reduced CLint significantly, from 6 to 3 l/hr/kg, although the decrease in CLint was not significant. The half decrease in CLint suggests a 50% decrease in the CYP3A activity of dogs. This extent was similar to those in hepatic microsomes from dogs treated with DEX in our previous study. Since QN is mainly metabolized by CYP3A [11] and is categorized as a drug with low hepatic extraction [2], our present study suggests that DEX treatment at clinical doses significantly decreases CYP3A activity, even in intact dogs, and thereby significantly alters the pharmacokinetics of the CYP3A substrate.

For drugs with low hepatic extraction like QN, a 50% decrease in CLint may lead to a 2-fold increase in the average drug concentration at the steady state. Therefore, great risk of adverse drug-drug interaction must be considered during DEX therapy if comedicated drugs have a relatively narrow therapeutic range. In fact, there are many reports describing such clinical adverse drug interactions by CYP3A inhibitors. Pohjola-Sintonen et al. [15] reported that itraconazole inhibited terfenadine metabolism and caused torsades de pointes, a serious ventricular arrhythmia. Corpier et al. [3] reported that cyclosporine inhibited lovastatin metabolism, and this caused acute renal failure and rhabdomyolysis in humans. Mullins et al. [13] reported that mibebradil interacted with dihydropyridine and beta-blocker metabolism, and this resulted in death and cardiogenic shock with intensive support of heart rate and blood pressure in humans. There is a possibility that such adverse drug-drug interactions may occur during DEX therapy.

In our previous study, we demonstrated that a clinical dose of DEX down-regulated the CYP3A subfamily, not only in dogs, but also in rats [21]. This fact may suggest that DEX down-regulates the CYP3A subfamily in animal species other than dogs and rats because therapeutic doses of this drug are similar among animal species and quite lower than those used in many reports describing CYP3A induction.

Although several researchers have reported induction of AGP by DEX in rats [18, 19, 16], our study indicated that
DEX decreased plasma AGP concentrations in dogs. Since QN is a basic drug, this decrease resulted in an increase in the $V_d$ and $CL_{tot}$ of QN by an increase in the unbound fraction of QN in the plasma. Because of these facts, the binding kinetics of QN was also examined in order to calculate $CL_{int}$, through which the significant inhibitory effect of DEX on CYP3A activity was observed in this study. As is seen in Fig. 1A and B, the plasma concentrations of the unbound form, which directly relate to pharmacological effects, also showed more evident accumulation in the body compared to the total plasma concentrations. The combined effects of decreased plasma protein binding and decreased unbound form, which directly relate to pharmacological effects, also showed more evident accumulation in the body compared to the total plasma concentrations. Therefore, more attention should be paid to possible drug-drug interactions caused by DEX under clinical conditions.

REFERENCES