



NOTE

Pharmacology

Interaction of cyclosporine with phenobarbital in cats: a preliminary study

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ABSTRACT. Phenobarbital (PB) decreases the cyclosporine (CsA) blood level in humans. However, the interaction of PB with CsA has not been reported in cats. This study investigated the effects of multiple doses of PB on the pharmacokinetics of CsA in three healthy cats. The treatments included oral CsA 5 mg/kg alone and oral CsA 5 mg/kg plus PB 5 mg/kg for 4 weeks. Co-administration of PB with CsA resulted in significant decreases in the oral bioavailability of CsA though both the first pass and elimination phases. These preliminary results suggest that oral administration of multiple doses of PB increases the required CsA dosage in CsA-based immunosuppressive therapy in cats.

KEY WORDS: cat, cyclosporine, pharmacokinetics, phenobarbital

Cyclosporine (CsA) is currently the core immunosuppressive drug for the prevention of allograft rejection after renal transplantation in cats [9]. In humans, CsA is a substrate of cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp) [6, 10, 23]. CYP is the most important enzyme family for drug metabolism in humans and other species. P-gp belongs to a subfamily of ATP-binding cassette transporters, and is involved in CsA excretion and acts as a drug efflux pump that actively transports CsA into the intestinal lumen [6, 12]. Therefore, the bioavailability of CsA could be affected by the co-administration of substrates of CYP and/or P-gp. Phenobarbital (PB) is the barbiturate used most commonly to treat seizures and epilepsy in dogs and cats [2]. PB is a strong CYP3A and P-gp inducer and is metabolized by CYP2C in hepatic microsomes in humans [20]. PB decreases blood CsA concentrations by inducing CYP and P-gp in humans [18]. In feline renal transplantation, an abrupt decline in blood CsA levels should be avoided because it will cause acute rejection. To our knowledge, however, there are no reports on the interaction of CsA with PB in cats. Therefore, this study evaluated the effects of PB on blood CsA levels in cats. We investigated the effects of administering multiple doses of oral PB on the pharmacokinetics of CsA in healthy cats. This study was classified as preliminary because of a small number of animals used.

Three healthy male cats were used in this study. Their weights ranged from 5.8 to 6.8 kg and their ages from 6 to 7 years old. Before this study, all cats were confirmed to be healthy based on a physical examination, complete blood count, biochemical profile, and urinalysis. Each cat underwent two treatments (A and B). In a clinical setting, CsA is administered a dosage of 3 to 5 mg/kg twice daily for renal transplantation in cats [5]. Therefore, the dose of CsA (Fine granules 17% [Mylan], Mylan, Tokyo, Japan) was adjusted to 5 mg/kg. The PB dose was about 5 mg/kg [mean dose 4.8 ± 0.4 (range 4.4–5.8) mg/kg, Phenovar[®] Tablets 30 mg, Fujinaga, Tokyo, Japan], which is reported to be sufficient for seizure control in cats [1, 4].

For treatment A, the cats received oral CsA alone. For treatment B, the cats were given 5 mg/kg PB once daily for 4 weeks and CsA 1 hr after the PB dose on day 28. The washout time between treatments was at least 1 month. The cats had access to water *ad libitum* throughout the study. A complete blood count, serum biochemical analysis, and urinalysis were repeated at the end of the study to detect any adverse effects of the drugs. This study was approved by the Iwate University Animal Care and Use Committee (A201337).

Two days before CsA administration, all three cats underwent brief anesthesia to place an indwelling 20-gauge 15-cm catheter (SMAC plus; Covidien, Shizuoka, Japan) in the right jugular vein. The catheter was maintained with daily flushing with heparinized saline until the experiment started. Whole blood samples were collected in dipotassium EDTA tubes through the catheter 0.5, 1, 2, 4, 6, 10, 12, and 24 hr after CsA administration. The catheter was flushed with heparinized saline after each sample collection. Then, the catheter was removed 2 days after the blood sampling. The blood samples were stored at -80°C

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until the measurements. The whole blood CsA concentration was measured using an electrochemiluminescence immunoassay by the commercial laboratory (SRL, Tokyo, Japan). According to the laboratory, the limit of quantitation of the whole-blood CsA concentration was 25 ng/ml.

The maximum blood concentration (C_{max}) and its corresponding time (t_{max}) were determined for each cat by plotting the blood CsA concentration versus time profile. The area under the curve from 0 to 24 hr (AUC_{0-24}) after CsA administration was calculated by the linear trapezoidal method. The terminal elimination rate constant (k) was calculated by linear least-squares regression analysis using the last three measurement points in the log-linear terminal phase. The $t_{1/2}$ was estimated to be $0.693/k$. The area under the first moment curve from 0 to 24 hr ($AUMC_{0-24}$) after CsA administration was also calculated by the linear trapezoidal method. The mean residence time (MRT) was calculated as $AUMC_{0-24}/AUC_{0-24}$. Differences in the pharmacokinetic parameters between each treatment were analyzed using the paired t -test and were regarded as statistically significant at $P < 0.05$. Each value is shown as the mean \pm SE.

Figure 1 shows the mean CsA blood concentration–time curves of the three cats. The C_{max} of the cats given repeated PB was 531.0 ± 126.0 ng/ml, approximately half the value in the cats treated with CsA alone ($1,266.0 \pm 183.0$ ng/ml); this difference was significant ($P < 0.05$). The AUC_{0-24} was $6,153.2 \pm 2,676.9$ ng/ml for treatment B and $14,026.7 \pm 3,096.0$ ng/ml for treatment A, and $t_{1/2}$ was 7.4 ± 0.8 and 14.8 ± 1.0 hr, respectively. Both values were significantly lower with treatment B ($P < 0.05$). The t_{max} was 4.7 ± 0.8 hr with treatment A and 6.0 ± 1.2 hr with treatment B, and did not differ significantly. MRT_{0-24} was 9.6 ± 0.1 hr with treatment A and 9.9 ± 0.7 hr with treatment B, and did not differ significantly. Table 1 lists the pharmacokinetic parameters for the three cats for each treatment.

In humans, the metabolism of CsA is mediated primarily by CYP, specifically CYP3A, in the liver and small intestine [6, 23]. PB is a CYP enzyme inducer [20] and can reduce blood CsA concentrations by inducing CYP enzymes, especially CYP3A [16, 22]. CYP3A has various isoforms and its tissue distribution is species dependent. In cats, two isoforms of CYP3A have been identified: CYP3A131 and CYP3A132 [7]. Both isoforms share the highest homology with canine CYP3A12, which is associated with CsA metabolism in dogs [13]. CYP3A131 transcript expression predominates in the liver and small intestine, with some expression in the brain and lungs [7]. Beusekom *et al.* [21] reported that hepatic microsomes in cats showed lower CYP3A activity than in dogs and humans. In feline renal transplant patients, ketoconazole reduces the dosage of CsA to approximately 60% of the total amount required to maintain the therapeutic CsA blood levels [14], which suggests that CsA metabolism is mediated partially by CYP3A in cats, as in humans. In cats, CYP3A also affects CsA metabolism, as in dogs and humans, despite its lower activity. Further investigation of the details of the effects of PB on CYP3A in cats is needed.

It has recently been shown that P-gp is involved in the pharmacokinetics of CsA [19, 24]. P-gp is a drug efflux pump that actively transports CsA into the intestinal lumen [6, 12], and CYP3A and P-gp have many common substrates [3]. In cats, P-gp was studied *in vitro* in feline lymphoma cells; the results suggested that the basic structure of the feline ortholog and its role in multidrug resistance were essentially the same as in other species [17]. To our knowledge, there is no study regarding whether PB can promote P-gp expression in intestine or liver. However, the long-term administration of PB and repeated seizures were reported to promote overexpression of P-gp in rat brain tissue [8]. These findings suggest that PB activates both the catalytic activity of CYP3A and the efflux function of P-gp, which may influence the pharmacokinetics of CsA in cats.

Based on our results, the administration of multiple oral doses of PB could decrease the blood concentration and AUC_{0-24} of CsA compared with CsA alone. In addition, $t_{1/2}$ differed statistically between CsA alone and CsA with PB. This implies that the administration of multiple doses of PB could decrease CsA blood level in cats, via both metabolism (including the first pass) and

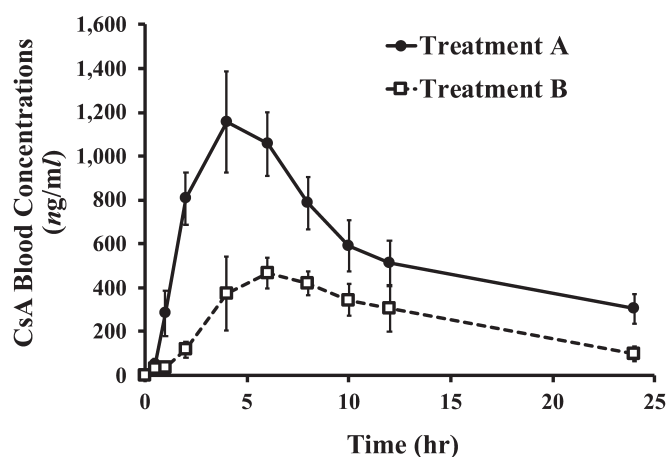


Fig. 1. Mean cyclosporine (CsA) blood concentration–time curves of the three healthy cats following treatment A (CsA alone) and treatment B [CsA + multiple therapeutic doses of phenobarbital (PB)]. Values are presented as means \pm SE.

Table 1. Effects of multiple oral doses of PB on the pharmacokinetic parameters of CsA in three healthy cats

Parameters	Treatment A (CsA alone)	Treatment B (CsA+PB)
AUC_{0-24} (ng•hr/ml)	$14,026.7 \pm 3,096.0$	$6,153.2 \pm 2,676.9^{a)}$
C_{max} (ng/ml)	$1,266.0 \pm 183.3$	$531.0 \pm 126.0^{a)}$
t_{max} (hr)	4.7 ± 0.8	6.0 ± 1.2
$t_{1/2}$ (hr)	14.8 ± 1.0	$7.4 \pm 0.8^{a)}$
$AUMC_{0-24}$ (ng•hr ² /ml)	$135,559.4 \pm 31,065.2$	$62,463.5 \pm 22,090.6$
MRT_{0-24} (hr)	9.6 ± 0.1	9.9 ± 0.7

Values are presented as means \pm SE. Treatment A: CsA alone. Treatment B: CsA + multiple doses of PB. a) Statistically different from treatment group A ($P < 0.05$).

the elimination phase in the pharmacokinetics of CsA, which is likely associated with CYP3A and P-gp activity.

Prevention of acute graft rejection correlates more strongly with AUC than trough level of cyclosporine in human solid organ transplant patients [11, 15]. Although monitoring of CsA trough level rather than AUC has been used in clinical feline renal transplantation, further study regarding relationship between AUC monitoring and clinical outcome of feline renal transplantation may be investigated in the future.

In conclusion, these preliminary findings show that PB significantly decreases the blood level of CsA in healthy cats and suggest that the co-administration of CsA with multiple oral doses of PB may require adjustment of the CsA dosage to prevent acute allograft rejection. A larger prospective study should explore the effects of PB on the CsA blood levels in cats.

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