Preliminary Study of Effects of Multiple Oral Dosing of Clarithromycin on the Pharmacokinetics of Cyclosporine in Dogs

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ABSTRACT. Clarithromycin (CLM) has been known to increase the cyclosporine (CsA) trough level in human and feline organ transplant patients. However, the interaction of CLM with CsA has not been reported in dogs. In this study, the effects of multiple dosing of CLM on the pharmacokinetics of CsA in three healthy beagles were investigated. The treatments included CsA 10 mg/kg alone and CsA 10 mg/kg + multiple-dose of CLM 10 mg/kg. Co-administration of CLM with CsA resulted in significant increases of oral bioavailability of CsA. The results of our study suggest that administration of multiple therapeutic doses of CLM may decrease the required CsA dosage in CsA-based immunosuppressive therapy in renal transplanted dogs.
KEY WORDS: clarithromycin, cyclosporine, pharmacokinetics, renal transplantation.

Cyclosporine (CsA), a substrate of cytochrome P450 (CYP) 3A and P-glycoprotein, is currently the primary immunosuppressive agent for the prevention of acute allograft rejection after renal transplantation in dogs and cats with end-stage kidney failure. In canine renal transplantation, CsA needs to be administered twice a day throughout the lifetime of the recipient [5]. Therefore, the cost of CsA-based immunosuppressive therapy is a major obstacle for this surgical procedure. Ketoconazole (Kcz) can inhibit both CYP3A and P-glycoprotein and has been used to reduce the dosage of CsA to avoid this concern [2, 3, 9]. However, the use of Kcz may induce hepatotoxicity which is known as it's side effect. If hepatotoxicity was emerged, Kcz needs to be removed from the immunosuppressive regimen, which may induce allograft rejection. It is known that clarithromycin (CLM) can also inhibit CYP3A and P-gp [17, 18, 20], and it appears to be less toxic than Kcz. It was reported that CLM significantly increased bioavailability of CsA and lowered the daily drug cost in both human lung and feline kidney transplant patients treated with CsA-based immunosuppressive therapy [10, 12]. Based on these findings, CLM may become an excellent alternative for Kcz for the canine renal transplant patients. In dogs, however, it has not been reported whether CLM affects the oral bioavailability of CsA.

The aim of this study was to evaluate the effect of CLM on the pharmacokinetic parameters of CsA in dogs. We investigated the effects of multiple oral administration of CLM at therapeutic doses on the pharmacokinetics of CsA in healthy Beagles.

Three healthy male dogs were used in this study. Their body weights ranged from 11.8 to 17.7 kg, and their ages ranged from 3 to 7 years old. Prior to this study, all dogs were confirmed to be healthy based on physical examination, complete blood count, biochemical profile and urinalysis. In a clinical setting, CsA is administered at a dosage of 10 mg/kg twice daily for renal transplantation in dogs [5]. Therefore, the dose of CsA (Ciclosporin fine granule 17% Mylan®, Mylan, Tokyo, Japan) was adjusted to 10 mg/kg. Two treatments (A and B) were performed in each dog. In treatment A, dogs received oral CsA alone. In treatment B, dogs were given about 10 mg/kg (therapeutic dose level) of CLM (Clarithromycin Capsule CH® 50 mg; Chouseido, Tokushima, Japan; range, 8.1–11.3 mg/kg; mean dose 9.9 mg/kg) twice daily for ten days (day 1 to day 10), and after the last administration, the dogs were fasted overnight with free access to water. The next morning, CLM was orally administered to the dogs and 2 hr later CsA orally. The washout times of each treatment were more than one month. This study was approved by the Iwate University Animal Care and Use Committee (A201234).

Whole blood samples were drawn through the cephalic vein at 0.5, 1, 2, 4, 6, 8, 12 and 24 hr after CsA administration and were collected in tubes containing EDTA. Blood samples were stored overnight at 0°C until measurements. Measurement of the whole blood CsA concentration was performed using a radioimmunoassay (RIA) with CYCLO-Trac SP-Whole Blood Cyclosporin kit (DiaSorin, Stillwater, MN, U.S.A.) by the commercial laboratory (SRL, Tokyo,

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Table 1. Effects of multiple doses of oral clarithromycin (CLM) on the pharmacokinetic parameters of cyclosporine (CsA) in healthy beagles (n=3)

Parameters	AUC_{0-24} (ng·hr/ml)	C_{max} (ng/ml)	T_{max} (hr)	$T_{1/2}$ (hr)	$AUMC_{0-24}$ (ng·hr^2/ml)	MRT_{0-24} (hr)
Treatment A	16,842 ± 3,052	1,533 ± 33	3.33 ± 0.67	12.72 ± 2.03	159,450 ± 42,255	9.21 ± 0.71
Treatment B	22,514 ± 3,223*	2,200 ± 231	3.33 ± 0.67	12.83 ± 1.47	207,742 ± 39,262*	9.12 ± 0.44

Values are presented as means ± SE. Treatment A: CsA (10 mg/kg PO) alone. Treatment B: CsA (10 mg/kg PO) + multiple therapeutic doses of CLM (10 mg/kg PO). * Statistically different from treatment group A (*P*<0.01).
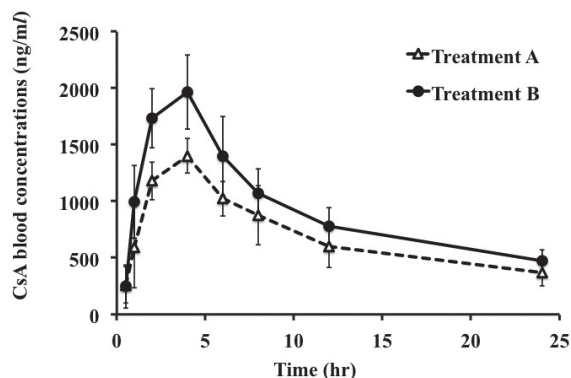

Fig. 1. Mean cyclosporine (CsA) blood concentration-time curves of healthy beagles following treatment A (CsA alone) and treatment B (CsA + multiple therapeutic doses of clarithromycin). Values are presented as means ± SE (n=3).

Japan). According to the commercial laboratory, the limit of quantitation in whole blood CsA concentrations was 4.0 ng/ml, and the linearity was seen between 20 and 700 ng/ml. The intra-day and inter-day assay precision were 2.24–5.97% and 4.06–9.02%, respectively.

The maximum blood concentration (C_{max}) and its corresponding time (t_{max}) were determined for each dog by observation of the blood CsA concentration versus time profile. The AUC from 0 to 24 hr (AUC_{0-24}) after a 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 four measurement points in the log-linear terminal phase. The $t_{1/2}$ was estimated as $0.693/k$. The area under the first moment curve from 0 to 24 hr ($AUMC_{0-24}$) after a CsA administration was also calculated by the linear trapezoidal method. The mean residence time (MRT_{0-24}) 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 to be statistically significant at *P*<0.05. Each value is shown as the mean ± SE.

The blood concentration-time curves after the two treatments are shown in Fig. 1. The AUC_{0-24} and $AUMC_{0-24}$ of dogs with CLM treatment (treatments B) were significantly higher than those of dogs treated with CsA alone (treatment A) (*P*<0.01). The average CsA blood concentrations in treatment B tended to be entirely higher than those of treatment

A and especially at 2 and 12 hr they were significantly higher (0.01<*P*<0.05). Pre-administration of multiple doses of CLM did not significantly affect C_{max}, t_{max}, $t_{1/2}$ and MRT_{0-24}. The pharmacokinetic parameters of CsA with or without CLM are listed in Table 1. All dogs showed no adverse effects associated with CLM, such as gastrointestinal irritation, during multiple CLM treatment.

CsA is known to be metabolized by CYP3A and P-glycoprotein (P-gp) in the liver and small intestine [22]. It was reported that as much as 50% of oral CsA metabolism may be attributed to intestinal metabolism in humans [6, 13]. When CsA is combined with the inhibitor of these proteins, CsA metabolism will be more or less affected. CLM, one of the 14 membered ring macrolides, has been known to inhibit both CYP3A and P-gp activities [8, 19, 21]. Based on the results of our pharmacokinetic study, administration of CLM could significantly increase the AUC_{0-24} of CsA compared to CsA alone. In addition, regarding $t_{1/2}$ and MRT_{0-24}, there were no statistical differences between CsA alone and CsA with CLM. This suggests that administration of multiple doses of CLM may increase oral bioavailability of CsA in dogs, mainly by decreasing the first-pass effect due to CYP3A and / or P-gp inhibition.

In human and feline transplantation, CLM decreased baseline CsA dose and resulted in successful reduction of treatment cost [10, 12]. Our results indicated that the use of CLM might reduce the dosage and cost as well as the administration frequency of CsA in dogs. This would be of clinical importance to make renal transplantation clinically acceptable for the owners. Although high doses of CLM may give rise to colic, inappetance, vomiting, diarrhea or other clinical signs of gastrointestinal irritation, this drug rarely induces significant hepatic or renal toxicity unlike antifungal agents, such as Kcz [14]. As far as used within therapeutic ranges, CLM may become an alternative option to Kcz for canine renal transplant patients requiring continuous immunosuppression through the life time.

Besides the effects of CLM therapy on CsA blood levels, macrolides are widely known to have direct or indirect effects on the immune system [1]. It was reported that CLM inhibited the production of IL-2 by human T lymphocytes in a dose-dependent manner, which may indicate that the immunosuppressive effects of CLM may be based partially on the ability to inhibit the production of IL-2 by T lymphocytes [17]. Co-administration of CLM and CsA may enhance the immunosuppressive effects on T lymphocyte proliferation developing to acute allograft rejection. CLM might be beneficial to canine kidney transplant recipients by

its immunosuppressive effects, thereby minimizing potential insults to the allograft.

CLM has been used primarily to treat respiratory tract infections in humans and is considered to be one of the safe drugs with fewer side effects [4, 11]. In dogs, CLM can be used as one of the therapeutic options for the treatment of severe or refractory leproid granuloma [14]. In canine kidney transplant patients, the occurrence of bacterial infections, such as upper respiratory tract infections and pneumonia, as a post-operative complication due to long term immunosuppression has been reported [5, 7, 17]. Hopper *et al.* reported that bacterial infections were considered as the cause of death in 23% of canine kidney transplant patients [7]. In healthy beagles, 10 mg/kg PO of CLM produced serum concentrations of approximately 3 μg/m*l*, although steady-state studies have not been performed in dogs [20]. Serum concentrations greater than 2 μg/m*l* are likely to be effective for conventional bacteria [15]. In this study, we used therapeutic doses of CLM 10 mg/kg PO twice daily. Thus, prophylactic use of CLM may be effective for the prevention of bacterial infectious complications in canine kidney transplantation. However, long-term use of CLM may induce the development of antibiotic resistant bacteria. Therefore, a further study is needed to investigate the effectiveness and safety of long-term CLM therapy for canine kidney transplantation.

In conclusion, the results of our study showed that CLM significantly increases the oral bioavailability of CsA in healthy beagles and suggest that co-administration of CsA with multiple oral dosing of CLM may replace the dose frequency from twice to once a day schedule. However, a larger scale, prospective study is warranted to investigate more extensively the impact of CLM on CsA in dogs, because of a small number of animals used in this study.

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