Effect of Multiple Oral Dosing of Fluconazole on the Pharmacokinetics of Cyclosporine in Healthy Beagles

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ABSTRACT. Fluconazole (Fcz) is successfully used in human organ transplant patients as an antifungal therapy. However, Fcz can increase the cyclosporine (CsA) trough level and lead to CsA nephrotoxicity. In canine renal transplantation, CsA has been used as a major immunosuppressant, and it is important to control its trough level. However, the interaction of Fcz with CsA has not yet been reported in dogs. In this study, the effect of Fcz treatment on the pharmacokinetics of CsA in four healthy beagles was investigated using a four-period crossover design. The treatments included CsA alone (A), CsA + multiple-dose Fcz 50 mg (B), CsA + multiple-dose Fcz 25 mg (C) and CsA + single-dose Fcz 50 mg (D). Blood CsA concentrations were measured at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hr after CsA administration. The AUC0–12 and Cmax values for treatment B were significantly higher than those for the other treatments. In particular, the AUC0–12 of treatment B was about two times higher than that of treatment A. Fcz administration did not significantly prolong the half-life or mean residence time of CsA. The results of our study show that administration of multiple therapeutic doses of Fcz can significantly increase the CsA blood concentration, which might partially depend upon the Fcz blood concentration. When Fcz is used in CsA-based canine renal transplantation, it may be necessary to adjust the CsA trough level by decreasing the dose.

KEY WORDS: Cyclosporine, fluconazole, pharmacokinetics.

Cyclosporine (CsA) is a lipophilic cyclic polypeptide with powerful immunosuppressive properties that is isolated from the fungus Tolypocladium inflatum. CsA specifically inhibits synthesis of interleukin (IL)-2, which induces activation and proliferation of T cells, and results in suppression of secondary synthesis of various cytokines, such as IL-4, interferon-γ and granulocyte-macrophage colony stimulating factor. In human medicine, CsA has been used widely to prevent allograft rejection after organ transplantation since its clinical introduction in 1978. More recently, CsA has also been shown to be effective for immune-mediated diseases, including chronic asthma, nephritic syndrome, psoriasis, Behcet’s disease and immune-mediated thrombocytopenia in children [1, 3, 8, 17, 28]. In veterinary medicine, CsA is very effective in preventing allograft rejection and prolonging graft survival time in feline renal transplant recipients [15, 16]. CsA has been reported to be effective in the treatment of canine atopic dermatitis, perianal fistula and keratoconjunctivitis sicca [11, 20, 23]. In several facilities, CsA has also been used as a core immunosuppressive drug in canine renal transplantation performed on animals with end-stage chronic renal failure [9, 21].

In human transplant recipients, systemic infection, as well as rejection episodes, is one of the important adverse effects associated with life-long immunosuppressive therapy. Fungal infections are not uncommon after renal transplantation, and systemic fungal infections are associated with high mortality in humans [6]. In human liver transplant patients, antifungal prophylaxis with fluconazole (Fcz) significantly reduces the incidence of invasive fungal infections [25]. Fcz penetrates the blood-brain, blood-prostate and blood-ocular barriers, and high concentrations are found in cerebrospinal fluid, urine and ocular fluids [14, 31]. Fcz shows efficacy against aspergillosis, candidiasis, coccidioidomycosis, cryptococcosis and histoplasmosis in animal models [10, 13, 26]. Although canine renal transplantation is still developing as a salvage treatment for end-stage chronic renal failure, systemic fungal infection should be considered a fatal complication of severely immunosuppressed conditions.

It is known that Fcz can inhibit cytochrome P450 (CYP) 3A, and it has less toxicity than ketoconazole, which has been widely used in small animal medicine. CsA is widely known as a substrate of CYP3A and P-glycoprotein. It has also been reported that Fcz significantly increases CsA bioavailability and may make it difficult to control CsA blood concentrations in human renal transplant patients treated with CsA-based immunosuppression [5, 30]. In humans, CsA nephrotoxicity is well known as a side effect, and this makes the therapeutic range of CsA quite narrow. CsA allograft nephropathy has also been recognized in dogs and cats [4, 22]. When the trough concentrations of CsA are
higher than the therapeutic range (400–600 ng/dl) in dogs, CsA toxicity may ultimately emerge [4, 21]. Therefore, it is critical to know about the interactions of CsA and Fcz in dogs when the latter is used for antifungal therapy in renal transplant recipients. However, it has not yet been reported whether Fcz can affect the oral bioavailability of CsA in dogs.

The aim of this study was to evaluate the effect of Fcz on the CsA blood level in dogs. We investigated the effects of multiple administration of oral Fcz at subtherapeutic or therapeutic doses compared with single oral administration of a therapeutic dose of Fcz on the pharmacokinetics of CsA in healthy Beagles.

MATERIALS AND METHODS

Four healthy female Beagles were used in this study. Their body weights ranged from 10.3 to 12.8 kg and their ages ranged from 1 to 3 years old. Before the start of this study, all dogs were confirmed to be healthy based on physical examination, complete blood count, biochemical profile (calcium, inorganic phosphate, creatinine, urea nitrogen, glucose, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and cholesterol) and urinalysis (dipstick, specific gravity and sediment). The dogs were randomly allocated to the four treatments in a four-period crossover design. In clinical canine renal transplantation, CsA is administered at a dosage of 10 mg/kg twice daily [9]. Therefore, the dose of CsA (ATOPICA soft gelatin capsules; Novartis Animal Health, Basel, Switzerland) was adjusted to approximately 10 mg/kg (range, 7.8 ± 9.7 mg/kg, according to capsule strength and dog weight; mean dose 8.58 ± 0.40 mg/kg). In treatment A, dogs received oral CsA alone. In treatment B, dogs were given Fcz at therapeutic dose levels (Fluconazon capsules; Nichi-iko, Toyama, Japan; range, 4.2–4.5 mg/kg; mean dose 4.3 ± 0.16 mg/kg) once daily for 10 days (days 1 through 10) followed by CsA 2 hr after the Fcz treatment on day 10. In treatment C, dogs were given Fcz at subtherapeutic doses (range, 2.0–2.3 mg/kg; mean dose 2.1 ± 0.13 mg/kg) once daily for 10 days (days 1 through 10) followed by CsA 2 hr after the Fcz treatment on day 10. In treatment D, dogs were given Fcz at therapeutic dose levels followed by CsA 2 hr after the Fcz treatment. The washout times after each treatment were >14 days. In this study, we selected 14 days as the washout period because of the half-life ($t_{1/2}$) of the drugs and on the basis of the results of other studies in which the washout period ranged from 7 to 14 days [2, 7, 14, 29].

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 stored in tubes with EDTA in a refrigerator (4°C) until measurements. Measurement of the whole blood CsA concentration was performed using a fluorescence polarization immunoassay using a TDx Cyclosporine A Dynapack kit (polyclonal; Abbott, Tokyo, Japan), which produces exact concentrations of CsA within 65–2000 ng/ml. When the CsA concentration was above the upper limit of detection of this test kit (>2,000 ng/ml), it was measured after diluting the blood samples twice with calibration solution A of the X Systems Cyclosporine and Metabolites Whole Blood Calibrators kit (Abbott, Tokyo, Japan).

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 area under the curve from 0–12 hr (AUC$_{0–12}$) after a CsA dose 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 points in the log-linear terminal phase. The $t_{1/2}$ was tentatively estimated as 0.693/$k$. The area under the first moment curve from 0–12 hr (AUMC$_{0–12}$) after a CsA dose was also calculated by the linear trapezoidal method. The mean residence time (MRT) was calculated as AUMC$_{0–12}$/AUC$_{0–12}$.

Differences in the pharmacokinetic parameters between each treatment were analyzed using the Tukey test and were regarded to be statistically significant at $P<0.05$. Each value is shown as the mean ± SD.

RESULTS

The blood concentration-time curves after the four treatments are shown in Fig 1. In this study, AUC$_{0–12}$ was calculated as an alternative to AUC$_{0–24}$ because the CsA blood concentration was below the limit of quantification at 24 hr in most cases (data not shown). Pre-administration of single or multiple doses of Fcz did not affect $t_{max}$. The mean whole blood concentrations of CsA for treatment B were constantly higher than those for treatment A. The $C_{max}$ of dogs with treatment B was significantly higher than that with treatments A, C or D. The AUC$_{0–12}$ of dogs with Fcz treatment (treatments B, C and D) was significantly higher than

![Fig. 1. Mean CsA blood concentration-time curves of the four healthy dogs following treatment A, CsA alone; treatment B, CsA+Fcz (multiple therapeutic doses); treatment C, CsA+Fcz (multiple subtherapeutic doses); or treatment D, CsA+Fcz (single therapeutic dose). Values are presented as means ± SD.](image)
that of dogs treated with CsA alone (treatment A; P<0.05). Especially in relation to treatment B, the AUC_{0-12} was about two times higher than that of treatment A. The AUC_{0-12} for treatment B was significantly different from that for treatments C and D. In regard to t_{1/2} and MRT, there were no significant differences between the CsA alone (treatment A) and Fcz treatments (treatments B, C and D). The pharmacokinetics of CsA are listed in Table 1. There were no significant differences in any parameters between treatments C and D.

DISCUSSION

Generally speaking, hepatic metabolism is considered to be of prime importance for drug absorption, with other areas of metabolism playing a comparatively minor role. However, intestinal metabolism may play a much greater role in the pharmacokinetics of orally administered drugs than previously thought. CsA is metabolized primarily by CYP, in particular CYP3A, in the liver and small intestine [12]. P-glycoprotein belongs to the subfamily of ATP-binding cassette transporters and is also associated with excretion of CsA, acting as a drug efflux pump that actively transports CsA back into the intestinal lumen [12]. As much as 50% of oral CsA metabolism may be attributed to intestinal metabolism [12]. Fcz produces inhibition of hepatic CYP3A in vitro [27]. On the other hand, Fcz seems to have no inhibitory effect on the P-glycoprotein encoded by MDR1 [27]. According to the results of this study, administration of multiple therapeutic doses of Fcz significantly increased the blood concentration and AUC_{0-12} of CsA by more than CsA alone and the other Fcz treatments. In addition, with regard to t_{1/2} and MRT, there were no statistical differences between CsA alone and the three Fcz treatments. This suggests that administration of multiple therapeutic, but not single or multiple subtherapeutic doses, of Fcz may increase oral bioavailability of CsA in dogs, mainly by decreasing the first-pass effect that is associated with CYP3A, rather than P-glycoprotein activity during intestinal absorption of CsA.

In our study, multiple therapeutic doses of Fcz (treatment B) significantly increased the AUC_{0-12} and C_{max} compared with the other Fcz treatments (treatments C and D). In human renal transplant patients, it has been reported that 200 mg Fcz, but not 100 mg, daily slowly increases the CsA concentration during therapy for 2 weeks, approximately doubling the CsA trough concentration [5, 18]. It has been reported that the serum concentrations of Fcz reach a plateau days 5–7 after oral administration of 400 mg Fcz for 31 days in patients with pulmonary cryptococcosis [24]. In addition, Fcz orally administered to dogs (10 mg/kg) had a t_{1/2} of 15 hr, and the peak plasma concentration was observed within 4 hr [14]. In this study, we did not monitor the blood Fcz concentration. However, we would have expected that continuous administration of therapeutic doses of Fcz for 10 days would have been sufficient for the Fcz blood level to stabilize at a higher level than that with single therapeutic or multiple subtherapeutic doses. When CsA is co-administered with Fcz, the increase in the blood CsA concentration may partially depend upon the blood Fcz concentration. However, the optimal concentration of Fcz to upregulate the CsA blood level in dogs is not clear.

In humans, nephrotoxicity is a known side effect of CsA, and this makes the therapeutic range of the CsA trough level quite narrow. In human renal transplant patients, CsA-induced acute nephrotoxicity is characterized histologically by necrosis and hyalinosis of smooth muscle cells in the afferent arterioles and/or isometric vacuolation of the proximal tubules [19]. These changes may be reversible by lowering the CsA dose, but allograft loss would develop if these histological changes continued to be widespread. CsA toxicity has also been reported in the renal allografts of dogs and cats with CsA trough levels above the therapeutic range [4, 22]. In our study, Fcz significantly increased the oral bioavailability and blood concentration of CsA. This suggests that the CsA trough level should be carefully monitored to keep it within the therapeutic range by adjusting the CsA dose when used with Fcz in dogs.

It has been reported that Fcz is well tolerated in humans, and the possibility of adverse events, such as nausea, other minor gastrointestinal problems and asymptomatic elevation of hepatic enzymes, is comparatively low [26]. Unlike ketoconazole, which is mainly metabolized in the liver, Fcz is primarily excreted unchanged in urine [14]. Although experience with this drug is limited in small animal practice,
no adverse effects have been reported in dogs during therapy. However, it may be beneficial to add a renal function test to the monitoring list, as well as the CsA trough level, when Fcz is applied to CsA-treated dogs.

In conclusion, the results of our study show that Fcz can significantly increase the oral bioavailability of CsA in healthy beagles and suggest that co-administration of CsA with Fcz may increase the CsA blood concentration. When CsA is co-administered with Fcz, patients should be carefully monitored during treatment. However, further studies on the effects of long-term treatment are needed before applying co-administration of Fcz and CsA to canine renal transplantation. When Fcz is administered as adjunctive therapy to CsA-treated dogs, as with renal transplant recipients, the CsA trough level, as well as renal function, should be closely monitored to check for CsA nephrotoxicity.

REFERENCES