BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **23**: 53–57 (2002)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/bdd.295

Minimal Effect of Ketoconazole on Cyclosporine (SangCyA™) Oral Absorption and First-Pass Metabolism in Rats: Evidence of a Significant Vehicle Effect on SangCyA Absorption

Susan Wong, Harrison T. Wong and Vincent J. Wacher*
AvMax Inc., 385 Oyster Point Blvd. # 9A, South San Francisco, CA, 94080, USA

ABSTRACT: The current work evaluated the effect of the CYP3A inhibitor ketoconazole on the oral absorption and first-pass metabolism of cyclosporine administered as the SangCyA formulation. Groups of 6 male Sprague–Dawley rats were administered SangCyA (5 and 15 mg/kg) by oral gavage alone and with ketoconazole (30 mg/kg). Blood cyclosporine levels were measured over 6 h, encompassing the cyclosporine absorption window. A significant vehicle effect on SangCyA absorption was observed. Comparing a 15 mg/kg dose, cyclosporine C_{max} (mean $\pm$ SD 1.12 ± 0.16 μg/ml) and AUC_{0-6} (5.34 ± 0.71 μg h/ml) were 50% lower when propylene glycol was used as gavage vehicle instead of saline (2.19 ± 0.94 μg/ml and 9.52 ± 2.52 μg h/ml, respectively). Coefficients-of-variation for these parameters were halved in the propylene glycol vehicle however T_{max} was unaffected. Ketoconazole increased cyclosporine C_{max} and AUC_{0-6} by 50–60%, regardless of the vehicle or the cyclosporine dose, without altering T_{max} (2–3 h). The small effect of ketoconazole suggests that CYP3A-mediated intestinal and first-pass hepatic metabolism are minor determinants of cyclosporine oral bioavailability in rats. Copyright © 2002 John Wiley & Sons, Ltd.

Key words: absorption; cyclosporine; ketoconazole; rats; vehicle

Introduction

Cyclosporine is an immunosuppressive agent used extensively to prevent the rejection of transplanted organs [1–3]. Cyclosporine is metabolized by cytochrome P450 3A (CYP3A) and clinical studies with the original Sandimmune® (SIM) and the more bioavailable Neoral® (NEO) formulations have shown that cyclosporine oral bioavailability can be improved 3–5-fold in patients and healthy volunteers by coadministration with the potent CYP3A inhibitor ketoconazole [4–6]. Dose-normalized cyclosporine C_{max} was increased 3–4-fold by ketoconazole in these subjects (consistent with increased absorption and/or inhibition of first-pass cyclosporine metabolism) and a detailed pharmacokinetic analysis indicated that up to two-thirds of the ketoconazole effect on cyclosporine oral bioavailability could be attributed to inhibition of cyclosporine metabolism in the small intestine [4,6]. Ketoconazole has also been shown to increase cyclosporine bioavailability in dogs [7], however it did not affect SIM oral absorption or first-pass metabolism in rats [8]. Cyclosporine is poorly metabolized by rat liver and intestinal microsomes *in vitro* [8,9] and the failure of ketoconazole to improve cyclosporine bioavailability in this species *in vivo* suggests that intestinal and first-pass metabolism by CYP3A

*Correspondence to: Ontogen Corporation, 6451 El Camino Real, Carlsbad, California, 92009, USA. E-mail: vwacher@worldnet.att.net

Received 11 September 2001
Revised 13 December 2001
Accepted 20 December 2001

may not be significant determinants of cyclosporine oral bioavailability in this species. An unaddressed concern from the previous rat study was whether the high cyclosporine dose (25 mg/kg) may have saturated metabolism *in vivo*, thus overcoming metabolic inhibition by ketoconazole (10 and 20 mg/kg).

The current report describes identical studies in rats measuring the effect of ketoconazole on cyclosporine oral absorption and first-pass metabolism using the SangCyA™ formulation (SANG). At the time of this work, SANG was marketed as a generic form of NEO [3,10], however it has since been withdrawn due to vehicle-dependent lack of bioequivalence [11]. There are limited published data describing SANG absorption in rats [12] and no reports of its potential interaction with ketoconazole in any species. To avoid the abovementioned concern over metabolic saturation, lower cyclosporine doses of 5 mg/kg (the clinical therapeutic dose) and 15 mg/kg are administered with a large excess of ketoconazole (30 mg/kg). Equivalent doses of ketoconazole resulted in up to 9-fold increases in levels of the CYP3A substrate K02 in the same species of rat [13]. Potential vehicle-dependence of the ketoconazole effect on cyclosporine oral bioavailability is evaluated by conducting parallel experiments using saline and propylene glycol as the gavage vehicle. A significant vehicle effect on SANG absorption is observed and its relevance to the withdrawal of this product is discussed.

Experimental

SangCyA™ cyclosporine oral solution, USP [modified] (100 mg/ml) (Sangstat Medical Corporation, Hayward, CA) was commercially available at the time of the study. Male Sprague–Dawley rats (250–300 g) with jugular vein cannulae were purchased from Harlan (Madison, WI). Animal dosing and blood sampling were conducted by Northview Pacific Laboratories Inc. (Hercules, CA) in accordance with the Animal Welfare Act. Rats were individually housed at 18–26°C and allowed free movement and access to water. Animals were fasted from 12 h prior to dose administration until study completion.

Separate groups of 6 rats were administered SANG (5 and 15 mg/kg) by oral gavage alone and with ketoconazole (30 mg/kg). Doses were prepared by mixing SANG (0.25 or 0.5 ml) with ethanol (0.4 ml) or a suspension of ketoconazole in ethanol (250 or 375 mg/ml) followed by dilution to the appropriate volume with either 0.9% saline or propylene glycol. Rats were administered 1.5 ml/kg of each dose using a standard gavage needle. Blood samples (500 µl) were taken prior to the dose and at 0.5, 1, 2, 3, 4 and 6 h post-dose through a jugular vein cannula. Samples were collected in Microtainer® tubes (Becton–Dickinson, Franklin Lakes, NJ) containing sodium EDTA anti-coagulant and were stored in the refrigerator prior to analysis. Blood volume was replaced after each draw by saline and cannula patency was maintained using saline–heparin. No precipitation or hemolysis of blood samples were observed over the course of the study.

Whole blood samples (400 µl) were extracted and analyzed by HPLC with UV detection as described previously [8]. Coefficients of variation for 100, 500 and 3000 ng/ml cyclosporine quality control samples (12 samples per concentration measured over 4 days) were 5, 4 and 3%, respectively, and all concentrations were within 5% of nominal values. Peak cyclosporine concentration (C_{max}) and time to achieve this concentration (T_{max}) were measured directly from concentration versus time profiles. Area under the concentration–time curve (AUC_{0-6}) was calculated using the linear trapezoidal method. Pharmacokinetic parameters for the different doses were compared using an unpaired *t*-test (normally distributed data) or the Mann–Whitney rank sum test.

Inhibition of cyclosporine metabolism by ketoconazole at the dose employed was confirmed *in vitro* by incubations with liver microsomes from a human donor and from dexamethasone-induced rats. Gavage emulsions from rat studies with a 15 mg/kg dose were diluted in methanol, providing stock solutions containing 1 mM cyclosporine alone or with 4.5 mM ketoconazole. Five µl of each stock solution was added to a 0.5 ml incubation mixture containing 200 µg/ml liver microsomal protein and 1 mM NADPH in 0.1 M phosphate buffer (pH 7.4). Final concentrations

of cyclosporine and ketoconazole in the incubation mixtures were 10 μM (a saturating substrate concentration) and 45 μM, respectively. Samples were incubated for 30 min then extracted and analyzed for cyclosporine and metabolites as described previously [8].

Results and Discussion

Consistent with clinical reports and published rat data, dose-normalized cyclosporine C_{max} and $AUC_{0–6}$ were 3–7-times higher for SANG (Table 1) than previously reported for SIM while T_{max} was reduced by 2–3 h [3,8,10,12]. SANG absorption was particularly sensitive to the gavage vehicle such that cyclosporine C_{max} and $AUC_{0–6}$ were reduced by 50% when SANG was administered in propylene glycol rather than saline (Figure 1). Coefficients of variation for these parameters were also halved in the propylene glycol vehicle. Coadministration of ketoconazole increased cyclosporine C_{max} and $AUC_{0–6}$ by 50–60%, regardless of the dose or the vehicle employed. T_{max} was not altered by vehicle or ketoconazole. Microsomal metabolism studies using diluted gavage emulsions found that ketoconazole completely eliminated cyclosporine metabolism in both rat and human liver microsomes even though cyclosporine was used at a saturating substrate concentration.

The small increase in cyclosporine absorption effected by ketoconazole in these animals is consistent with our previous finding that lower ketoconazole doses (10 and 20 mg/kg) had no effect on cyclosporine absorption or first-pass metabolism from the SIM formulation (25 mg/kg) [8]. The ketoconazole concentration in the current gavage emulsions (38 mM) was 54 000-times higher than its reported K_i (0.7 μM) [14], indicating ketoconazole levels were more than sufficient to inhibit cyclosporine metabolism *in vivo*. The effect of ketoconazole was not different for the 5 and 15 mg/kg cyclosporine doses, however the large excess of ketoconazole and its potency as a CYP3A inhibitor suggest that this does not arise from saturation of metabolism at

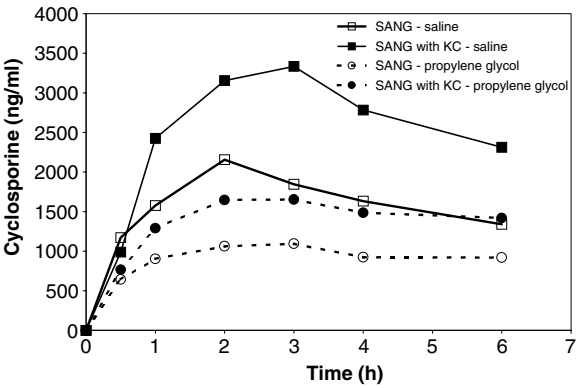

Figure 1. Mean cyclosporine concentrations vs time profiles (n=6) for rats administered SANG (15 mg/kg) alone or with ketoconazole (KC; 30 mg/kg) using saline or propylene glycol as the gavage vehicle

Table 1. The effect of ketoconazole on cyclosporine oral absorption in rats[a]

Parameter	SANG dose		
	5 mg/kg	15 mg/kg	15 mg/kg
Gavage vehicle	Saline	Saline	Propylene glycol
C_{max} (μg/ml)	0.84 ± 0.30 (36)	2.19 ± 0.94 (43)	1.12 ± 0.16 (14)[e]
+ketoconazole[b]	1.25 ± 0.26 (21)[c]	3.53 ± 0.91 (26)[c]	1.71 ± 0.17 (10)[d,e]
T_{max} (h)	1 (1–8)	2 (2–3)	3 (2–6)
+ketoconazole[b]	4 (2–8)	2 (1–3)	3 (2–4)
$AUC_{0–6}$ (μgh/ml)	4.95 ± 1.85 (37)	9.52 ± 2.52 (26)	5.34 ± 0.71 (13)[e]
+ketoconazole [b]	8.09 ± 1.05 (13)[c]	15.29 ± 3.80 (25)[c]	8.30 ± 1.09 (13)[d,e]

[a]Data are mean ± SD (CV%) except T_{max} which are median (range).
[b]30 mg/kg.
[c]Statistically different from control $P<0.05$.
[d]Statistically different from control $P<0.001$.
[e]Statistically different from saline vehicle $P<0.005$.

Table 2. Comparison of cyclosporine formulations

Formulation	Excipients
Sandimmune® (SIM)	Cyclosporine (100 mg/ml), alcohol (12.5% vol/vol); olive oil, Labrafil M 1944 CS (polyoxyethylated oleic glycerides)
Neoral® (NEO)	Cyclosporine (100 mg/ml); alcohol (11.9% vol/vol); corn oil-mono-di-tri-glycerides, polyoxyl 40 hydrogenated castor oil NF, DL-α-tocopherol USP, propylene glycol USP
SangCyA™ (SANG)	Cyclosporine (100 mg/ml); alcohol (10.5% vol/vol); propylene glycol USP and polysorbate 80 NF

the cyclosporine doses used. Published reports demonstrate poor metabolism of cyclosporine by rat liver microsomes [8] and negligible cyclosporine metabolism by rat small intestinal microsomes *in vitro* [9]. The relatively small ketoconazole effect on cyclosporine bioavailability observed in this study is consistent with poor intestinal and first-pass hepatic metabolism of cyclosporine in rats *in vivo*. In the absence of significant metabolism, even complete inhibition of CYP3A by ketoconazole (as indicated by the *in vitro* data) will only result in a small increase in cyclosporine oral bioavailability. Ketoconazole is also a modest inhibitor of the intestinal drug efflux transporter P-glycoprotein (P-gp) [13,15]. The high ketoconazole dose employed in the current study may partly inhibit P-gp-mediated cyclosporine efflux in the intestinal tissue of these animals, and this may also contribute to the small increase in cyclosporine levels.

The significant reduction in SANG absorption when administered in propylene glycol bears further discussion. One explanation for this effect is sequestration of the lipophilic cyclosporine in the propylene glycol vehicle, thus preventing its partitioning into the aqueous phase adjacent to the intestinal wall. A similar effect of lipid vehicle on cyclosporine absorption was not observed in our previous study, where corn oil did not alter the pharmacokinetics of the SIM formulation [8]. This discrepancy suggests that simple sequestration of cyclosporine in a lipid vehicle is not the predominant explanation for SANG vehicle sensitivity. A comparison of excipients used in the different cyclosporine formulations is provided in Table 2. SANG is significantly more water soluble than SIM and has substantially higher oral bioavailability. The mechanism for increased SANG solubility has not been published, however it may involve formation of microemulsions as observed for the NEO formulation [10]. Decreased SANG absorption in the presence of excess propylene glycol most likely arises from disruption of the critical excipient interactions responsible for increased cyclosporine aqueous solubility and/or intestinal permeability. Despite a reduction in absolute cyclosporine levels, the variability in cyclosporine pharmacokinetic parameters was lower when administered in propylene glycol rather than saline, presumably due to more uniform dissolution of the highly lipophilic cyclosporine in the more lipid-like vehicle.

In conclusion, the small effect of high-dose ketoconazole on cyclosporine levels in rats suggests that CYP3A-mediated first-pass metabolism in the intestine and liver are not significant determinants of cyclosporine oral bioavailability in this species. Rats may not be a useful preclinical model in which to evaluate metabolic drug interactions with cyclosporine. The vehicle effect on SANG absorption is of some interest in light of the withdrawal of this product [11]. SANG is bioequivalent to NEO when both are administered in chocolate milk, a more lipid-like vehicle, but they are not bioequivalent in the aqueous vehicle apple juice [3,11]. The reason for this difference has not been reported, however the current findings suggest that interactions of SANG, and potentially NEO, with the more lipid-like chocolate milk vehicle may significantly alter cyclosporine absorption. Further studies are required to elucidate the mechanism of such interactions.

References

1. Fahr A. Cyclosporin clinical pharmacokinetics. *Clin Pharmacokinet* 1993; **24**: 472–495.

2. Friman S, Backman L. A new microemulsion formulation of cyclosporin: pharmacokinetic and clinical features. *Clin Pharmacokinet* 1996; **30**: 181–193.
3. Schroeder TJ, Cho MJ, Pollack GM, *et al*. Comparison of two cyclosporine formulations in healthy volunteers: bioequivalence of the new Sang-35 formulation and Neoral. *J Clin Pharmacol* 1998; **38**: 807–814.
4. Gomez DY, Wacher VJ, Tomlanovich SJ, Hebert MF, Benet LZ. The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine. *Clin Pharmacol Ther* 1995; **58**: 15–19.
5. Foradori A, Mezzano S, Videla C, Pefaur J, Elberg A. Modification of the pharmacokinetics of cyclosporine A and metabolites by the concomitant use of Neoral and diltiazem or ketoconazole in stable adult kidney transplants. *Transplant Proc* 1998; **30**: 1685–1687.
6. Wu C-Y, Benet LZ, Hebert MF, *et al*. Differentiation of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporine. *Clin Pharmacol Ther* 1995; **58**: 492–497.
7. Dahlinger J, Gregory C, Bea J. Effect of ketoconazole on cyclosporine dose in healthy dogs. *Vet Surg* 1998; **27**: 64–68.
8. Wacher VJ, Wong S, Wong HT. Peppermint oil enhances cyclosporine oral bioavailability in rats. Comparison to d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and ketoconazole. *J Pharm Sci* 2002; **91**: 77–90.
9. Whalen RD, Tata PNV, Burckart GJ, Venkataramanan R. Species differences in the hepatic and intestinal metabolism of cyclosporine. *Xenobiotica* 1999; **29**: 3–9.
10. Kovarik JM, Mueller EA, van Bree JB, *et al*. Cyclosporine pharmacokinetics and variability from a microemulsion formulation—a multicenter investigation in kidney transplant patients. *Transplantation* 1994; **58**: 658–663.
11. Henney JE. From the Food and Drug Administration: nationwide recall of SangCya oral solution. *JAMA* 2000; **284**: 1234.
12. Cho MJ, Levy RE, Pouletty PJ. Oral cyclosporin formulations. *US patent* 5,766,629. 1998.
13. Zhang Y, Hsieh Y, Izumi T, Lin ET, Benet LZ. Effects of ketoconazole on the intestinal metabolism, transport and oral bioavailability of K02, a novel vinylsulfone peptidomimetic cysteine protease inhibitor and a P450 3A, P-glycoprotein dual substrate, in male Sprague–Dawley rats. *J Pharmacol Exp Ther* 1998; **287**: 246–252.
14. Pichard L, Fabre I, Fabre G, *et al*. Cyclosporin A drug interactions. Screening for inducers and inhibitors of cytochrome P-450 (cyclosporine A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metab Dispos* 1990; **18**: 595–606.
15. Takano M, Hasegawa R, Fukuda T, Yumoto R, Nagai J, Murakami T. Interaction with P-glycoprotein and transport of erythromycin, midazolam and ketoconazole in Caco-2 cells. *Eur J Pharmacol* 1998; **358**: 289–294.

