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Report

High-Fat Meals Increase the Clearance of Cyclosporine

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High-fat meals increase the clearance and volume of distribution, but not the mean residence time, of cyclosporine in seven healthy volunteers when either plasma or blood analyses are used. This unexpected finding for a low-extraction ratio drug should not be explainable in terms of increased blood flow. We hypothesize that dietary fat can act as a carrier for cyclosporine and enhance both its volume of distribution and its clearance. We suggest that clearance then occurs following metabolism of lipids due to lipase activity within the hepatocyte, thereby releasing free drug for metabolism.

KEY WORDS: cyclosporine; clearance; food effect; lipase.

INTRODUCTION

Most studies of the effect of food on drug pharmacokinetics have concentrated on changes in drug absorption and bioavailability, which have been shown to be generally unpredictable, with food causing all conceivable alterations in bioavailability (i.e., decreased, delay, increase, and no change) depending upon the specific drug and the meal type (1). The effect of food on the absorption of cyclosporine (CYA) has been investigated by Ptachcinski *et al.* (2), who reported a 1.5-fold increase in the relative bioavailability of CYA when CYA was administered with breakfast using blood analysis. In contrast, Keown *et al.* (3) first reported that food reduces the absorption of CYA but later reported that they could not confirm these findings (4). In 1988, Keogh *et al.* (5) reported that food had no significant effect on the absorption of CYA in cardiac transplant recipients. We recently reported a 1.8- and 2.6-fold increase in the relative bioavailability of CYA, when the drug was administered together with a high-fat breakfast to healthy volunteers (6) using blood and plasma analyses, respectively.

Very few food effect studies have examined the consequence of meals on drug disposition by carrying out a parallel study in which the meal effect is examined following intravenous drug dosing. This is necessary, however, to discriminate whether a change in area under the concentration–time curve (AUC) results from altered bioavailability, altered drug disposition, or a combination of both. Olanoff *et al.* (7) have demonstrated conclusively that food increases the clearance of intravenous propranolol, a high-extraction ratio drug, by 38%, which corresponds well with a 34% increase in estimated hepatic blood flow, measured by indocyanine green clearance. Thus, the 67% increase in bioavailability noted following propranolol oral dosing with a meal is greater than the AUC increase observed, since clearance also increases. In this paper we describe a marked increase in cyclosporine clearance following i.v. dosing together with a high-fat meal. Since cyclosporine is a low- to moderate-extraction ratio drug, for which blood flow is usually not a major determinant of clearance, we did not expect to see such an increase. Rather we had thought that a decrease in clearance was more likely due to increased concentrations of binding substances following a high-fat meal.

METHODS

The present study was carried out in seven healthy volunteers (three male and four female). The detailed study protocol and methods will be described in a full report of the pharmacokinetics of CYA in healthy volunteers (8). The two intravenous doses described here constitute two legs of a four-phase study over 6 months in which healthy volunteers received oral (10 mg/kg) and intravenous infusion (4 mg/kg over 2.5 hr) doses of CYA while fasting and with high-fat meals. On all occasions volunteers were given a high-carbohydrate, low-fat vegetarian meal 16 hr before CYA administration and fasted until the next morning. The first intravenous infusion was begun in the fasted state and volunteers received 100 ml of chocolate milk (the vehicle used for oral dosing). For the next 2.5 hr the subjects were allowed one 100-ml drink of decaffeinated tea or coffee. At the end of the 2.5-hr infusion, volunteers were given a breakfast consisting of fruits and fruit juices. The second intravenous infusion was also begun together with 100 ml of chocolate milk after a 16-hr fast. However, in this case the volunteers received a high-fat vegetarian breakfast consisting of two scrambled eggs in butter, hash brown potatoes, buttered toast, and orange juice just after the start and while receiving their infusion. Six hours after beginning the infusion, the volunteers received a high-fat lunch which included cheese pasta, french fried potatoes, buttered toast, and cheese cake. Eleven hours after the infusion began the volunteers received a high-fat dinner, identical to the lunch described above.

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Blood samples (14 ml) were drawn at 17 times over a 24-hr period. Both blood and plasma (separated at 37°C) were analyzed for CYA by HPLC (9). The area under the curve (AUC) was calculated using the log trapezoidal rule. Clearance (CL) was calculated by dividing the dose by the corresponding AUC. Mean residence time (MRT) and volume of distribution at steady state (V_{ss}) were calculated using noncompartmental methods (10), correcting for the infusion time.

RESULTS

Semilogarithmic plots of plasma concentration time data following intravenous CYA infusions in one volunteer are depicted in Fig. 1. Mean values (±SD) of CL, MRT, and V_{ss} for CYA in plasma are given in Table I under low-fat/fasting and high-fat conditions. The increases in CL and V_{ss} were significant at $P < 0.002$ and $P < 0.003$, respectively, using Student's paired t test and occurred in all seven volunteers. No significant difference in MRT was found as a function of the high-fat meal. Increases in CL and V_{ss} for blood concentration measurements were significant but not as great as that observed for plasma concentrations due to the high nonlinear red blood cell-to-plasma partition coefficient for this drug (11).

DISCUSSION

Very few food effect studies have examined the consequence of meals on drug disposition. In one case where food appears to alter propranolol disposition, the effect was attributed to increased CL due to an increase in hepatic blood flow for this high-extraction ratio drug.

We noted a marked increase in CYA bioavailability with a high-fat meal (6) and speculated initially that this may be partially due to a decrease in CL of CYA when a high-fat meal is given. This speculation appeared reasonable since CYA is a low- to moderate-extraction ratio drug and therefore the fraction free in the blood or plasma should be an important consideration. In plasma CYA binds 40–55% to high-density lipoproteins (HDL), 24–31% to low-density lipoproteins (LDL), and 4–10% to very low-density lipoproteins (VLDL) and chylomicrons, with only 4–7% unbound (12). High-fat meals should increase the total lipid concentration and, consequently, decrease the free fraction and, we speculated, therefore, decrease CL.

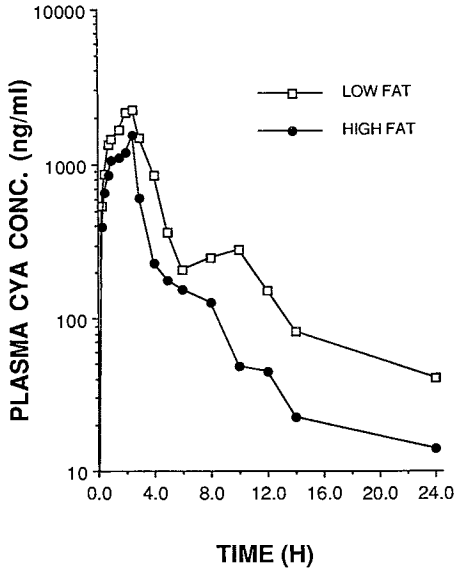

Fig. 1. Plasma cyclosporine concentration–time profile obtained in a representative subject, following a 2.5-hr intravenous infusion (4 mg/kg) of cyclosporine with and without high-fat meals.

As noted in Fig. 1 and Table I, we observed the exact opposite of what we had speculated. We observed an increase in CL and V_{ss} in both blood and plasma. The CL increase was 49% in plasma and 22% in blood. The decision as to whether blood or plasma monitoring is most suitable for CYA monitoring is a matter of controversy. We will address this question and our belief that the plasma measurements are more relevant in a future publication.

As stated above, we had speculated prior to carrying out this study that a high-fat meal may lead to increased binding and a decreased CYA free fraction. Such a change was expected to lead to a decreased CL and possibly a decreased V_{ss}. In contrast, opposite results were obtained. The increase in V_{ss} may be explained as follows. CYA is a highly lipophilic drug and binds extensively to lipoproteins, which are cleared relatively quickly by the adipose tissue. We postulate that due to the increase in lipoprotein concentration following high-fat meals, CYA is being carried along with the lipids into the adipose tissue and thereby increasing the V_{ss}. Contribution of LDL as a carrier for CYA has also been hypothesized by DeGroen (13), which supports our observation of an increase in V_{ss} with high-fat meals.

The increase in CL may also be explained as a result of increased transport across cell membranes. Generally, drugs bind to proteins which are too large to cross the cell membrane and which are metabolized relatively slowly as compared to the drug. Highly lipophilic drugs are highly bound to various fractions of lipoproteins, and various classes of lipoproteins are metabolized at different rates. CYA binds preferentially to HDL as compared to VLDL, yet the metabolism of VLDL and chylomicrons (70–90% triglycerides) is faster relative to that of HDL (3–5% triglycerides). LDL is intermediate in terms of binding and metabolism rates. Dietary fat, which consists mainly of VLDL and chylomicrons, should alter the distribution of CYA bound to various classes of lipoproteins (14). The chylomicrons are catabolized by the enzymes in the adipose tissue to chylomicron remnants and

Table I. Mean (±SD) Pharmacokinetic Parameters Estimated from Plasma Cyclosporine Concentrations Following Intravenous Infusions (4 mg/kg) of Cyclosporine to Seven Healthy Volunteers With and Without High-Fat Meals

Parameter	Fasting/ low fat	High fat	Significance
CL (L/hr/kg)	0.47 (0.10)	0.70 (0.16)	$P < 0.002$
MRT (hr)	2.53 (0.09)	2.71 (0.42)	NS[a]
V_{ss} (L/kg)	1.18 (0.25)	1.85 (0.28)	$P < 0.003$

[a] Not significant ($P = 0.4$).

these remnants are taken up by the hepatocytes via endocytosis and are metabolized. CYA bound to LDL can also be transported into the hepatocytes via hepatic LDL receptors. We hypothesize that metabolism of these chylomicron remnants and LDL causes the release and increased concentration of free CYA within the hepatocyte. Depending upon the relative rates of metabolism and diffusion, the drug will either be metabolized or diffuse out of the cell to establish a new equilibrium. Cefalu and Pardridge (15) showed that the rate of diffusion of CYA is slow, so it is quite probable that once the CYA has been transported into the hepatocyte, it will be metabolized rather than diffuse out of hepatocyte. Fabre *et al.* (16), using rabbit hepatocytes, also concluded that the rate of CYA diffusion is very poor.

If the hypothesized scenario described above does occur, then the net observed effect will be an increase in CL despite increased binding and decreased fraction unbound. In other words the CL of such a drug will be affected by both the fraction unbound and the concentration of the readily metabolized binding protein. One study in the literature appears to contradict the above hypothesis. Lithell *et al.* (17) showed that the CL of CYA was inversely correlated with the lipoprotein concentrations across a population of uremic patients. However, two distinctions should be pointed out. First, in the present study CL changes were determined using each subject as his/her own control with respect to increased lipoprotein concentrations, versus attempting to correlate CL with lipoprotein concentrations across a population as per Lithell *et al.* (17). Second, uremic patients exhibit decreased lipase activity, adding an additional variable in the patient population which is not present in our study. However, both our work and that of Lithell and co-workers suggest that a portion of the high variability observed for CYA pharmacokinetics may be due to changes in blood lipoprotein concentrations.

Our results contradict those observed by Wassef *et al.* (18), who examined the effect of concomitant infusion of a fat emulsion (Intralipid, 10%) on CYA disposition in five dogs. These authors report that significantly increased triglyceride concentrations (1.43 ± 0.19 vs 0.62 ± 0.06 mM) resulted in a nonsignificant 28% increase in CYA plasma AUCs measured from 2 to 10 hr using a nonspecific RIA assay. Note that the increased nonspecific AUC in dogs is exactly opposite the effect described here. In our study mean triglyceride concentrations increased from 0.95 ± 0.27 to 1.31 ± 0.23 mM, while mean cholesterol concentrations decreased from 4.60 ± 0.84 to 3.71 ± 0.36 mM in going from the low-fat meal study to the high-fat meal. We have also carried out a nonspecific TDX assay of the plasma samples obtained following intravenous dosing and observe only a mean 2.6% nonsignificant difference in AUCs for the same samples yielding a 49% highly significant difference when unchanged CYA is measured. We believe that the dog study by Wassef *et al.* (18) using a nonspecific assay is not predictive of the effects of a high-fat meal on unchanged CYA pharmacokinetics in man. It is likely that the contradictory results previously reported concerning food effects are a function of assay method. Ptachcinski *et al.* (2), who reported increased CYA absorption, used a specific HPLC assay, while Keogh *et al.* (5) reported no food effect using a nonspecific method. We will further address the relevance of nonspecific assays in a future publication.

In conclusion, a high-fat meal increased the CL and V_{ss} of CYA in healthy volunteers. It is probable that CL and distribution of lipophilic drugs like CYA might be enhanced with dietary fat, which consists mainly of VLDL and may be reduced by increases in HDL. If our hypothesis is correct, then one might also expect CL of CYA to vary as a function of lipase activity. We now plan to study the effect of various lipid concentrations on CL of CYA using liver perfusion techniques.

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REFERENCES

1. P. G. Welling. *Clin. Pharmacokinet.* 9:404–434 (1984).
2. R. J. Ptachcinski, R. Venkataramanan, G. J. Burckart, J. A. Gray, D. H. Van Thiel, A. Sanghvi, and T. Rosenthal. *Transplantation* 40:174–176 (1985).
3. P. A. Keown, C. R. Stiller, A. L. Laupacis, W. Howson, R. Coles, M. Stawecki, J. Koegler, G. Carruthers, N. McKenzie, and N. R. Sinclair. *Transplant. Proc.* 14:659–661 (1982).
4. P. A. Keown, C. R. Stiller, N. R. Sinclair, G. Carruthers, W. Howson, M. Stawecki, J. McMichael, J. Koegler, N. McKenzie, and W. Wall. *Transplant. Proc.* 15:2438–2441 (1983).
5. A. Keogh, R. Day, L. Critchley, G. Duggin, and D. Baron. *Transplant. Proc.* 20:27–30 (1988).
6. S. K. Gupta and L. Z. Benet. *Clin. Pharmacol. Ther.* 35:148 (1989).
7. L. S. Olanoff, T. Walle, T. D. Cowart, U. K. Walle, M. J. Oexmann, and E. C. Conradi. *Clin. Pharmacol. Ther.* 40:408–414 (1986).
8. S. K. Gupta, R. C. Manfro, S. J. Tomlanovich, J. G. Gambertoglio, M. R. Garovoy, and L. Z. Benet. Submitted.
9. S. K. Gupta and L. Z. Benet. *J. Liq. Chromatogr* 12:1451–1462 (1989).
10. L. Z. Benet and R. L. Galeazzi. *J. Pharm. Sci.* 68:1071–1074 (1979).
11. B. Legg and M. Rowland. *Pharm. Res.* 5:80–85 (1988).
12. S. K. Gupta. *Pharmacokinetic and Interaction Studies of Cyclosporine in Renal Transplant Patients.* Ph.D. thesis, University of Manchester, Manchester, UK, 1987.
13. P. C. De Groen. *Mayo Clin. Proc.* 63:1012–1021 (1988).
14. B. Legg, S. K. Gupta, and M. Rowland. *Ther. Drug Monit.* 10:20–27 (1988).
15. W. T. Cefalu and W. M. Pardridge. *J. Neurochem.* 45:1954–1956 (1985).
16. G. Fabre, P. Bertault-Peres, I. Fabre, P. Maurel, S. Just, and J. Cano. *Drug Metab. Dispos.* 15:384–390 (1987).
17. B. Lithell, B. Odlind, A. Selinus, A. Lindberg, B. Lindstrom, and L. Frodin. *Transplant. Proc.* 18:50–51 (1986).
18. R. Wassef, S. Cohen, and B. Langer. *Transplantation* 41:266–268 (1986).