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The effect of food and bile acid administration on the relative bioavailability of cyclosporin

ANDERS LINDHOLM[1,2], STAFFAN HENRICSSON[1] & RUNE DAHLQVIST[1]
Departments of [1]Clinical Pharmacology and [2]Transplantation Surgery, Huddinge Hospital, Huddinge, Sweden

1 The relative bioavailability of cyclosporin was studied in 11 healthy volunteers after single oral capsule doses of cyclosporin on three separate occasions; fasting, with breakfast and with breakfast together with bile acid tablets (400 mg of cholic acid and 100 mg of dehydrocholic acid).

2 There was a significant increase in the area under the blood concentration *vs* time curve (AUC) of cyclosporin when the drug was taken together with breakfast and bile acid tablets (9078 ng ml^{-1} h) as compared with breakfast alone (7453 ng ml^{-1} h, $P < 0.05$) or fasting conditions (7283 ng ml^{-1} h, $P < 0.01$).

3 A blood drug concentration *vs* time curve displaying two peaks was present in 9/11 subjects when cyclosporin was taken with breakfast or with breakfast and bile acid tablets, but only one peak was present when cyclosporin was taken during fasting, suggesting an enterohepatic circulation of cyclosporin or a second absorption phase after the meal.

4 In a separate study, 12 h trough blood cyclosporin concentrations were measured before and after 1 week of bile acid treatment in 19 clinically stable, out-patient transplant recipients who were treated with oral cyclosporin solution (mean dose 2.0 mg kg^{-1} twice daily). The administration of cyclosporin was not standardized with regard to food intake. There was no significant difference in the blood concentrations of cyclosporin before and after bile acid treatment (114 ± 38 ng ml^{-1} *vs* 121 ± 38 ng ml^{-1}).

5 The absorption of CyA is incomplete and variable and depends on several factors such as gastric emptying, intestinal motility, bile composition and flow and concurrent medication. It is concluded that under strictly standardized conditions oral bile acid supplementation will moderately enhance cyclosporin absorption.

Keywords cyclosporin bioavailability bile acids

Introduction

The bioavailability of cyclosporin has been reported to vary from 2 to 89% *between* patients (Bertault-Pérès *et al.*, 1985; Burckart *et al.*, 1986; Frey *et al.*, 1988; Ptachcinsky *et al.*, 1985a). Under standard conditions, the area under the plasma drug concentration *vs* time curve (AUC) may vary up to two-fold *within* healthy individuals (Lindholm *et al.*, 1988). This intra- and inter-individual variability in bioavailability complicates cyclosporin therapy, as a low value may result in graft rejection, while an unnecessarily high value may result in adverse effects (Irschik *et al.*, 1984; Klintmalm *et al.*, 1985; Lokiec *et al.*, 1983; Rogerson *et al.*, 1986). Factors that govern intra- and interpatient variation in the bioavailability of cyclosporin need to be clarified.

The effect of simultaneous intake of food on

Correspondence: Dr Anders Lindholm, Department of Clinical Pharmacology, Huddinge Hospital, S-141 86 Huddinge, Sweden

the absorption of cyclosporin is controversial. Thus, food has been reported to enhance (Ptachcinski *et al.*, 1985b) or to be without a significant effect (Keogh *et al.*, 1988; Keown *et al.*, 1983) on the absorption of the drug. However, the absorption of cyclosporin has been found to depend on bile production and flow (Andrews *et al.*, 1985; Ericzon *et al.*, 1987; Mehta *et al.*, 1988). Therefore, the administration of bile acids might improve the absorption of cyclosporin and thereby reduce variation in its bioavailability.

We have studied the influence of a light breakfast on the relative bioavailability of cyclosporin in healthy volunteers. In addition, the effect of exogenous bile acid administration was studied in healthy volunteers and in renal transplant recipients.

Methods

Volunteer study

Eleven men, aged 24 to 41 years (mean age 28 years) and with weights from 66 to 93 kg, participated as volunteers after giving informed consent. All subjects were healthy as assessed by medical history and routine laboratory examination. The study was approved by the local ethics committee and the Department of Drugs at the Swedish National Board of Health and Welfare.

Soft gelatine capsules of cyclosporin (25 and 100 mg, Sandoz Ltd, Basel, Switzerland), were given as single doses of about 6 mg kg^{-1} body weight (ranging from 5.84 to 6.07 mg kg^{-1}) on three separate occasions (set A, B, C). A minimum period of 7 days separated each dose.

In a random order, cyclosporin was given at 08.00 h in the fasting state (set A), together with breakfast (set B) or together with breakfast and bile acid tablets (set C). The intake of food and fluid was standardised during the first 8 h of each study period.

In set A, the capsules of cyclosporin were ingested with 150 ml of water. Thereafter, the subjects fasted for 4 h. In set B, cyclosporin was given together with a standard light breakfast (one cup of coffee or tea, two pieces of toast, 10 g of butter and one glass of juice). Set C was identical to set B, except that the subjects who received bile acid tablets (total dose of 400 mg of cholic acid and 100 mg of dehydrocholic acid; Fellesan, Pharmacia, Sweden). An identical lunch was served on all three occasions, 4 h after the intake of cyclosporin. The dose of bile acids was chosen to approximate the amounts normally excreted within 1 h (B. Angelin, personal communication).

Venous blood samples were taken prior to and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 15, 24 and 32 h after the dosage using EDTA as anticoagulant. The whole blood samples were stored at −20° C until assay.

Patient study

Nineteen out-patients, aged 23 to 67 years, who had received kidney transplants at least 4 months previously, and who had stable renal function and an unchanged dosage of cyclosporin for at least 2 weeks, were included in this study. The patients were instructed to keep to their normal habits but the timing and composition of meals was not standardised. Cyclosporin in olive oil solution was administered orally 12-hourly (at a daily dose ranging from 0.96 mg kg^{-1} to 6.16 mg kg^{-1}, mean 4.02 ± 1.42 (s.d.) mg kg^{-1} day^{-1}), and the dosage remained unchanged throughout the study. Whole blood samples were collected on days −1 and 0 for the measurement of morning trough cyclosporin concentrations. On day 0, the patients started medication with bile acid tablets (200 mg of cholic acid and 50 mg of dehydrocholic acid twice daily) together with the dose of cyclosporin twice daily for 8 days. On days 6 and 7, samples were again collected for the measurement of morning 12 h trough cyclosporin concentrations. The mean concentrations prior to and during medication with bile acid tablets were compared.

Analyses

In the volunteer study, the whole blood samples were assayed for cyclosporin by high performance liquid chromatography, using a modification of the method described by Shibata *et al.* (1987). The following modifications were made. To 500 μl whole blood was added 500 μl of 0.15 mM dextran sulphate sodium salt solution containing 75 mM $MgCl_2$. Cyclosporin D (250 ng) was added as internal standard. The samples were extracted for 15 min with 3 ml of n-hexane, followed by centrifugation. The organic phase was evaporated to dryness under nitrogen and the residue was dissolved in 100 μl 5% v/v ethanol in n-hexane and injected into the chromatograph.

A 4.6 × 150 mm Supelcosil column was used, with 3 μm silica particles. The mobile phase comprised 10% v/v ethanol in n-hexane, at a flow rate of 1.2 ml min^{-1}. The column was maintained at 50° C to prevent peak broadening. An integrator (HP3390A, Hewlett Packard,

Paramus, Waldwick NJ, USA) was used to record the signal from the u.v. detector, which was operated at 210 nm.

The intra- and interassay coefficients of variation were 3 ($n = 20$) and 7% ($n = 22$), respectively, at a cyclosporin concentration of 200 ng ml^{-1}.

All samples were assayed in duplicate, and the mean results were used in the pharmacokinetic calculations.

The area under the whole blood drug concentration *vs* time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity using the value of the terminal elimination rate constant (calculated from 15–32 h data).

The samples from the patient study were analysed in duplicate by radioimmunoassay utilising monoclonal antibodies specific for parent cyclosporin (Quesniaux *et al.*, 1986, 1987) according to the instructions supplied with the kit (Sandoz Ltd., Basle, Switzerland, 1987). Pipetting and incubation was performed at 2–3° C. Charcoal treatment was performed with protein-coated Norit A charcoal (United States Biochemical Corporation, Cleveland, Ohio, USA). Optiphase Safe (LKB, Bromma, Sweden) was used as the scintillation fluid. The method had intra- and inter-assay coefficients of variation of 10% ($n = 18$) and 12% ($n = 27$), respectively, at a cyclosporin concentration of 125 ng ml^{-1}; and 6% ($n = 18$) and 7% ($n = 26$), respectively, at a concentration of 485 ng ml^{-1}. There was close correlation between results obtained with the RIA and h.p.l.c. methods (RIA-method = 3.4 + 1.07 × h.p.l.c. $r = 0.97$, $n = 152$).

Statistics

A two-way analysis of variance with a randomized block design was used for the multiple comparison between the data sets and a *t*-test was used for the pair-wise comparison between two data sets. A P value of less than 0.05 was used as the level of statistical significance. Values are given as mean ± standard deviation (s.d.).

Results

Volunteer study

The statistical analysis showed that there was no period effect related to the order of treatments. Mean and individual blood drug concentration-time curves are shown in Figures 1 and 2, respectively.

Area under the blood drug concentration vs *time curve*

The mean total AUC was 7283 ng ml^{-1} h for set A (fasting conditions), 7453 ng ml^{-1} h for set B (breakfast) and 9078 ng ml^{-1} h for set C (breakfast and bile acid tablets; Table 1). There was a significant difference in a multiple comparison between the data sets ($P < 0.05$, two-way analysis of variance). In the pair-wise comparisons performed between the data sets we found a significant difference between sets A and C (25% increase, $P < 0.01$) and between sets B and C (22% increase, $P < 0.05$), but not between sets A and B (2% increase, NS). The mean extra-

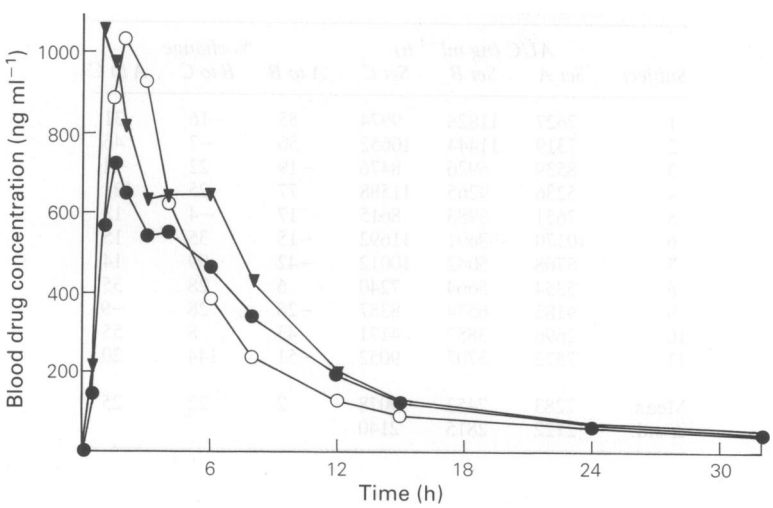

Figure 1 Mean blood drug concentration *vs* time profiles in eleven subjects after dosage of cyclosporin in the fasting state (○), together with breakfast (●) or together with breakfast and bile acid tablets (▼).

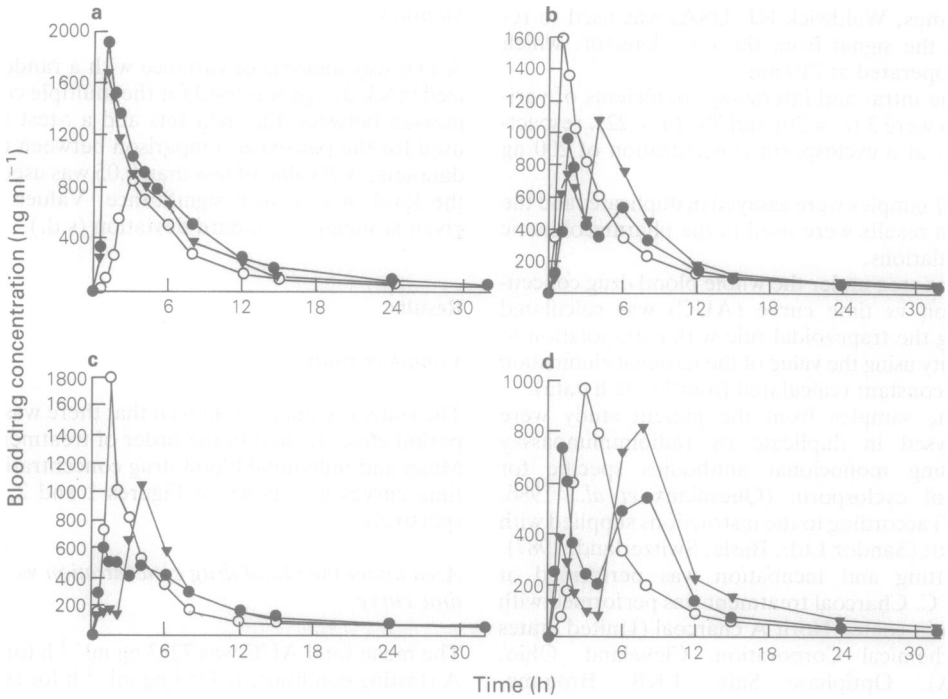

Figure 2 Individual blood drug concentration *vs* time profiles after dosage of cyclosporin in the fasting state (○), together with breakfast (●) or together with breakfast and bile acid tablets (▼) in subject nos 1, 3, 6 and 8.

Table 1 Individual areas under the concentration-time curve (AUC) of cyclosporin when the drug was taken during fasting (set A), together with breakfast (set B) or together with breakfast and bile acid tablets (set C)

	AUC (ng ml^{-1} h)			*% change*		
Subject	*Set A*	*Set B*	*Set C*	*A to B*	*B to C*	*A to C*
1	7627	11828	9974	55	−16	31
2	7319	11444	10652	56	−7	46
3	8539	6926	8476	−19	22	−1
4	5236	9265	11588	77	25	121
5	7651	8983	8615	17	−4	13
6	10170	8691	11692	−15	35	15
7	8768	5042	10012	−42	99	14
8	5354	5664	7240	6	28	35
9	9183	6574	8387	−28	28	−9
10	2696	3857	4171	43	8	55
11	7572	3707	9052	−51	144	20
Mean	7283	7453	9078	2	22	25
± s.d.	2122	2815	2140			

Table 2 Pharmacokinetic parameters (mean ± s.d. or median and range) of cyclosporin after oral intake during fasting conditions (set A), together with breakfast (set B) or together with breakfast and bile acid tablets (set C) in 11 healthy subjects

	Set A	*Set B*	*Set C*
AUC (ng ml^{-1} h)	7283 ± 2122	7453 ± 2815	9078 ± 2140[a,b]
Median time to peak blood concentration (h)	2 (1–4)	1.5 (1–6)	1.5 (1–8)
Peak blood concentration (ng ml^{-1})	1139 ± 401	942 ± 429	1274 ± 547
Terminal elimination half-life (h)	17.8 ± 7.2	13.0 ± 3.9[c]	14.5 ± 8.7

a) $P < 0.01$ *vs* set A
b) $P < 0.05$ *vs* set B
c) $P = 0.05$ *vs* set A

polated areas were 15.8%, 10.5% and 11.8% for set A, B and C, respectively.

During fasting conditions (set A) there was a more than 3-fold interindividual variation in the AUC (from 2513 ng ml^{-1} h to 8240 ng ml^{-1} h. Intersubject variability did not differ between the study periods (*F*-test; the coefficients of variation being 0.29, 0.38 and 0.24 for set A, B and C, respectively). The two subjects with the smallest AUC in set A had the highest increase in set C (subjects 4 and 7, with increases in AUC of 55% and 127%). However, in general, there was no significant correlation between the AUC associated with fasting and the percentage change from set A to set C ($r < 0.2$).

Other pharmacokinetic parameters

Individual values of the time to peak blood drug concentration ranged from 1.0 to 8.0 h. There was no difference in the median time to peak concentration between the sets, with median values ranging from 1.5 to 2.0 h (Table 2). Individual values of peak blood drug concentration varied from 304 ng ml^{-1} to 2447 ng ml^{-1}. The mean values did not differ significantly between the treatments (Table 2).

A second peak in the blood drug concentration *vs* time plot was not present in any subject in set A, but was apparent in 9/11 subjects in both sets B and C. This difference was highly significant ($P < 0.01$, binomial test). In patients with two peaks in the concentration-time profiles (Figure 2), the second peak was higher than the first in 3/9 subjects in set B and in 4/9 subjects in set C. This second peak appeared at 6.4 ± 2.6 h and 5.5 ± 1.6 h after dosage, respectively, for sets B and C (NS). The corresponding concentrations were 596 ± 230 ng ml^{-1} and 765 ± 218 ng ml^{-1}, respectively (NS).

The terminal elimination half-life in blood ranged from 8 to 39 h with a median of 13 h. The mean value tended to be longer in set A (17.8 ± 7.2) than in B (13.0 ± 3.9; $P = 0.05$, Table 2).

The usefulness of blood concentrations at defined time points as predictors of the AUC was studied for samples collected at 6, 12 and 24 h after dosage. For all 33 sets of observations the correlation coefficients between AUC and the cyclosporin concentration at the respective time points were 0.80 at 6 h, 0.70 at 12 h and 0.64 at 24 h after dosage.

Patient study

Of the nineteen patients who received bile acid tablets for 1 week, fourteen patients had increased, one had unchanged and four had decreased blood cyclosporin concentrations (Table 3). The mean change was +6% (from 114 to 121 ng ml^{-1}). This difference was not statistically significant. Two patients had diarrhoea at the end of the study week.

Discussion

Cyclosporin is highly lipophilic and for oral use it is dissolved in olive oil (oral solution) or maize oil (capsules). Its absorption is dependent on bile acid secretion as shown in liver transplant recipients, in whom external drainage of bile resulted in poor absorption of the drug (Andrews *et al.*, 1985; Mehta *et al.*, 1988). It is likely that cyclosporin is absorbed after incorporation into bile micelles. Ingestion of food (or administration of exogenous bile acids) stimulates bile acid production and gall bladder contraction. Therefore, it might be expected that simultaneous intake of food may enhance the absorption of cyclosporin.

The aim of our study was to assess the influence of food and bile acid tablets on the absorption of cyclosporin under conditions that would be applicable directly to the clinical

Table 3 12 h trough blood concentrations of cyclosporin before and after 1 week of bile acid treatment in 19 cyclosporin-treated renal transplant recipients (mean of two determinations on two consecutive days)

	Cyclosporin (ng ml^{-1})		
Patient	*Before*	*During*	*Mean change*
1	154	236	53
2	52.5	152	190
3	91	91	0
4	95	110	16
5	138	135	−2
6	132	99.5	−25
7	165	95.5	−42
8	98	111	13
9	143	151	6
10	110	119	8
11	105	132	26
12	118	124	5
13	110	118	7
14	72.5	75	3
15	55.5	60.5	9
16	116	124	7
17	103	109	6
18	93	96	3
19	209	168	−20
Mean	114	121	6
s.d.	38	38	

setting. Therefore, the bile acid tablets were not given alone but with a light meal, as most patients take cyclosporin with breakfast. As the exposure of healthy volunteers to cyclosporin should be limited, we did not give a reference intravenous dose of cyclosporin. Therefore only relative bioavailabilities were compared.

The AUC of cyclosporin increased by only 2% after a light breakfast compared with the fasting state. This finding is in accordance with that of Keogh *et al.* (1988), who studied heart transplant recipients at between 3 and 20 months after transplantation. In contrast, Ptachcinski *et al.* (1985b) found a mean 60% increase in the AUC of cyclosporin when taken with food. The latter study was performed in renal transplant recipients within 2 weeks after transplantation. One explanation for their finding may be that the decreased production and flow of bile postoperatively in the fasting state is antagonized by food. It is also likely that, if present, any effect of food on cyclosporin absorption will depend on the composition of the diet.

To our knowledge, the effect of bile acid treatment on cyclosporin absorption has not been reported. With simultaneous administration of bile acid tablets, the AUC of cyclosporin increased by 24% compared with the fasting state, and by 22% compared with the breakfast period, indicating a direct effect of bile acid tablets on the absorption of cyclosporin.

In the group of patients treated with bile acid tablets for 1 week no significant increase in trough cyclosporin concentration occurred. The discrepancy in the results obtained in patients and volunteers may have several explanations. An important difference in experimental design was that meals and habits were standardized in the volunteers but not in the patients. Also, the patients received an oral solution of cyclosporin while the volunteers received capsules. Furthermore, dosage in the patients was about 1/3 of that given to the volunteers (mean 2.01 mg kg^{-1} *vs* 6.00 mg kg^{-1}). There is some evidence that the absorption of cyclosporin is zero-order in rats (Ueda *et al.*, 1983, 1984) and man (Grevel *et al.*, 1986; Reymond *et al.*, 1988). Finally the relationship between AUC and trough drug concentration at 12 h was weak ($r = 0.70$ in the volunteers), even though this was taken into account by using the mean of the determinations on two consecutive days and by studying 19 patients.

Double peaks in the blood drug concentration *vs* time curves have been observed previously (Kahan *et al.*, 1983; Lindholm *et al.*, 1988; Phillips *et al.*, 1988). These may be an effect of either a second absorption phase (e.g. increased absorption rate following the mid-day meal) or of enterohepatic circulation of cyclosporin or both. Enterohepatic circulation of cyclosporin has been proposed not to occur, as only trace amounts of parent cyclosporin (< 0.1%) were found in the bile (Venkataramanan *et al.*, 1985). However, we have recently found a sulphate conjugate of cyclosporin that is excreted in the bile (Henricsson *et al.*, 1989). This metabolite (and other conjugates) may be cleaved by intestinal bacteria or enzymes in the gut wall and re-enter the circulation as parent drug.

In contrast to the absence of a second peak in the blood drug concentration time curves in *fasting* patients in the present study, we observed a second blood drug concentration peak after intake of cyclosporin during fasting conditions in a previous study (Lindholm *et al.*, 1988). However, in the previous study, cyclosporin was ingested with 150 ml of chocolate milk, while in the present study cyclosporin was ingested with water only. This difference may be sufficient to cause a differential effect on gall bladder emptying.

We conclude that bile acid administration, but not a light breakfast, increases the AUC of

cyclosporin when given as capsules to healthy volunteers. This suggests that bile acid formation is an important determinant of the absorption of cyclosporin. However, continuous bile acid administration did not increase the trough blood cyclosporin concentration significantly in renal transplant recipients created with cyclosporin solution. Finally, the data suggest that cyclosporin may undergo enterohepatic circulation, although a second absorption phase after meals cannot be excluded.

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