# Influence of a Fat-Rich Meal on the Pharmacokinetics of a New Oral Formulation of Cyclosporine in a Crossover Comparison with the Market Formulation

**Edgar A. Mueller,[1] John M. Kovarik,[1,5] Johannes B. van Bree,[2] Joachim Grevel,[3] Peter W. Lücker,[4] and Klaus Kutz[1]**

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The influence of a fat-rich meal on the pharmacokinetics of cyclosporine from a new oral formulation (Sandimmune Neoral) was compared in a randomized, four-way crossover study to the currently marketed formulation (Sandimmune) in 24 healthy male volunteers. Single oral doses of 300 mg Sandimmune and 180 mg Sandimmune Neoral were each administered once under fasting conditions and once 30 min after starting a high-fat meal. Serial blood samples were obtained over a 48-hr period after each administration, and whole-blood cyclosporine concentrations were determined by a specific monoclonal radioimmunoassay method. Food had a marked effect on cyclosporine absorption from Sandimmune manifested by a nearly doubled time to reach the peak concentration and a 37% increase in the area under the curve. This was associated with significant elevations in subsequent whole-blood cyclosporine concentrations compared to the fasting administration. For Sandimmune Neoral the influence was less pronounced. The maximum concentration was decreased by 26%, without a relevant change in the time to reach the peak; the area under the curve showed a slight reduction of 15%. The relatively minor influence of a fat-rich meal on the absorption of cyclosporine from Sandimmune Neoral is advantageous when individualizing a dosage regimen under clinical and outpatient administration conditions.

KEY WORDS: cyclosporine; food effect; pharmacokinetics; formulation.

## INTRODUCTION

Cyclosporine is an immunosuppressive agent widely used in organ transplantation and more recently in autoimmune diseases. Its absorption pharmacokinetics from the currently marketed oral formulation (Sandimmune) are known to exhibit a high variability (1). One factor which may contribute to this variability is the time of drug administration in relation to meals; however, the exact influence of food on the pharmacokinetics of cyclosporine remains controversial. Investigations in different transplant patient populations and in healthy volunteers have observed no significant effect (2–4) or enhanced absorption (5,6). A possible explanation for these conflicting results is different fat content of the food consumed. While fat-rich meals increased the bioavailability of cyclosporine (6), meals with a normal- or low-fat content did not influence the extent of absorption (3,4,6).

Recently, a new oral formulation (Sandimmune Neoral) was developed which incorporates the drug in a microemulsion preconcentrate containing a surfactant, lipophilic and hydrophilic solvents, and ethanol. Preliminary studies in animals and humans demonstrated a more consistent absorption profile from the new formulation.[6] This crossover investigation was undertaken to determine the influence of a high-fat meal on the pharmacokinetics of cyclosporine after oral administration of Sandimmune Neoral in comparison to Sandimmune. A fat-rich meal was chosen to assess the formulations under dietary conditions which are known to influence the absorption of cyclosporine from the currently marketed formulation (6).

## METHODS

### Subjects

Twenty-four healthy male volunteers aged 27 ± 4 years (mean ± standard deviation) and weighing 71.5 ± 7.9 kg were enrolled after granting written informed consent. Volunteers were evaluated for general good health on the basis of medical history, physical examination, electrocardiogram, hepatitis and HIV serologies, and routine biochemical and hematologic tests.

### Study Design

The study protocol was approved by a local medical ethics committee and was performed in accordance with the Declaration of Helsinki. The study was conducted as a randomized four-treatment, four-period crossover investigation with successive administrations separated by a washout phase of 2 weeks. Treatments A and B consisted of a single 300-mg oral dose of the reference cyclosporine formulation (three 100-mg soft gelatin capsules of Sandimmune, Sandoz Pharma, Ltd.; Lot No. Y 144 0890), and Treatments C and D of a single 180-mg oral dose of the test cyclosporine formulation (three 60-mg soft gelatin capsules of Sandimmune Neoral, Sandoz Pharma, Ltd.; Lot No. X 177 0991). Each formulation was given once under fasting conditions with 200 mL tap water (Treatments A and C) and once with a fat-rich meal (Treatments B and D). The doses were chosen based on the results of a previous bioavailability study in fasting healthy volunteers to provide similar exposure (area under the curve) for the reference treatments (A and C).

Subjects were confined to the study center from 36 hr before until 48 hr after each drug administration. They received an identical meal the evening before dosing and fasted from 12 hr before until 4 hr after drug administration with the

[1] Department of Human Pharmacology, Drug Safety Assessment, Sandoz Pharma, Ltd., 4002 Basle, Switzerland.
[2] Biopharmaceutics Department, Sandoz Pharma, Ltd., Basle, Switzerland.
[3] Biomedical and Agricultural Services of Texas, Inc., Austin, Texas.
[4] Institute for Clinical Pharmacology, Grünstadt, Germany.
[5] To whom correspondence should be addressed.

[6] Sandoz Pharma, Inc., Investigator's Brochure: Sandimmun Neoral in Transplantation, Basle, Switzerland, 1992.



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exception of Treatments B and D, when the drug was administered 30 min after starting a high-fat breakfast (total caloric content, 960 kcal; fat content, 54.4 g). Beginning 4 hr after drug administration in all four treatments, subjects were given standardized, scheduled meals which were identical for all subjects on all dosing days. Venous blood samples for the determination of cyclosporine in whole blood were obtained predose and then 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, and 48 hr after drug administration. Samples were collected in EDTA-containing tubes, gently inverted several times, and frozen at −20°C.

### Bioanalytical Methods

Concentrations of cyclosporine A in whole blood were assayed using the commercially available Sandimmune radioimmunoassay (Sandoz Ltd., Basle, Switzerland) which is based on the use of a monoclonal antibody specific for the parent compound (7). At quality control concentrations of 6.25, 12.5, 100, 400, and 1600 ng/mL, the respective accuracy was 2.5, −0.7, −11.0, −7.2, and 3.1%; the intraassay coefficient of variation was 28.9, 19.8, 8.2, 7.1, and 10.5%; and the interassay coefficient of variation was 7.6, 10.2, 11.4, 10.1, and 5.4%. The overall detection limit calculated from the mean concentration corresponding to 95% binding was 5.5 ± 0.9 ng/mL ($n$ = 17).

### Pharmacokinetic Evaluation

Noncompartmental pharmacokinetic characteristics were derived by standard methods. The maximum whole-blood ($b$) concentration $C_{max,b}$ and the time of its occurrence $t_{max}$ were compiled from the concentration–time data. The area under the curve $AUC_b$ was calculated by the linear trapezoidal rule to the last blood concentration $C(t_z)_b$ above the limit of detection and extrapolated to infinity by the addition of the term $C'(t_z)_b/\lambda'_z$, where $C'(t_z)_b$ and $\lambda'_z$ are the predicted concentration at time $t_z$ and the terminal elimination rate constant determined by nonlinear regression analysis. The extrapolations contributed on average 2.1% (range, 0.6 to 5.4%) to the total $AUC_b$.

To characterize the absorption phase better, the equation for a two-compartment, open pharmacokinetic model with zero-order input and first order elimination from the central compartment (8,9) was fitted to the concentration–time data by iterative, weighted nonlinear regression analysis (10). The observed concentrations were weighted as the reciprocal of the analytical variance. For profiles exhibiting two peaks, a disposition model comprising the superposition of the concentration–time profiles following two successive zero-order input steps was applied (9). In addition to the lag time ($t_{lag}$) and the duration of apparent zero-order input ($T$) for characterizing the absorption phase, the half-lives of the initial disposition ($t_{1/2\lambda 1}$) and terminal ($t_{1/2}$) phases were derived as ln(2) divided by the respective rate constants $\lambda_1$ and $\lambda_z$. For profiles exhibiting two input steps, the second absorption phase was described by $t_{lag,2}$ and $T_2$.

### Statistical Evaluation

The pharmacokinetic characteristics of cyclosporine from the four treatments were assessed for normality of distribution (Wilk–Shapiro test) and homogeneity of variances (Levine test). Depending on the outcome of these tests, the parameters were compared by analysis of variance (ANOVA) or Friedman's nonparametric ANOVA. Significance was subsequently allocated based on Newman-Keuls post hoc multiple-comparisons test or Friedman's multiple-comparisons test. Statistical hypotheses were tested at the 0.05 significance level. All statistical calculations were performed using the RS/1 software package (11).

The presence or lack of influence of a fat-rich meal on cyclosporine pharmacokinetics was also assessed as an equivalence problem (12). This approach, however, is based on statistical methods for a 2-period crossover design. In the absence of period effects and with an explorative intention, it was applied to these data to provide a statistical assessment of the food effects which could be related to an accepted measure of clinical relevance (i.e., bioequivalence standards). For each formulation, $t_{max}$, $C_{max,b}$, and $AUC_b$ obtained under fasting and fed conditions were compared by a distribution-free analysis including 90% confidence intervals (13). For $C_{max,b}$ and $AUC_b$ the multiplicative model (logarithmic transformation) was used, for which the point estimate is the parameter ratio (fed/fasting) · 100. For $t_{max}$ the additive model was used, with the point estimate expressed as the absolute difference of fed minus fasting (hours). Accordingly, lack of influence was concluded for $C_{max,b}$ and $AUC_b$ if the 90% confidence interval was in the equivalence range 80 to 125% and for $t_{max}$ if the 90% confidence interval was within −0.5 to 0.5 hr for the reference formulation and −0.3 to 0.3 hr for the test formulation (i.e., ±20% of the respective fasting mean $t_{max}$'s). This procedure ensures that the risk of erroneously accepting lack of influence is at most 5% (13). For comparative purposes, the difference of the point estimate from 100% (multiplicative model) or from 0 hr (additive model) was used to quantify the magnitude of a food effect on each formulation.

## RESULTS

### Clinical Observations

All four treatments were well tolerated. There were no clinically relevant or drug related changes in vital signs or electrocardiograms. Biochemical and hematologic tests showed no relevant difference in any parameter between reference and test treatments.

### Pharmacokinetics of Cyclosporine and Comparison of Formulations

Whole-blood cyclosporine concentration-time data were well described using a two-compartment open pharmacokinetic model with one or two apparent zero-order input steps and first-order elimination from the central compartment. The agreement between the two pharmacokinetic analyses was assessed by comparing the $AUC_b$ estimates from the noncompartmental analysis with those derived from the compartmental parameters (i.e., $AUC_b$ = Dose/$[(V_{c,b}/f) \cdot k_{10}]$, where $V_{c,b}/f$ is the apparent volume of the central compartment and $k_{10}$ is the elimination rate constant from the central compartment) for each formulation. The

lines of identity and regression were virtually superimposed in both cases: slope = 0.978, $r^2$ = 0.990, for the reference formulation and slope = 0.956, $r^2$ = 0.994, for the test formulation.

The pharmacokinetic characteristics of cyclosporine after each treatment are compiled in Table I. Geometric mean whole-blood concentration–time profiles for the four treatments are compared graphically in Fig. 1. Under fasting conditions, cyclosporine appeared in blood after an initial lag time of 25 min for both formulations. Blood concentrations subsequently increased more rapidly for the test formulation as evidenced by a shorter $t_{max}$ ($P < 0.05$) and duration of apparent zero-order input ($T$) and by a higher $C_{max,b}$ ($P < 0.01$) compared to the reference formulation. Under fasting conditions, 14 profiles exhibited two absorption steps after administration of the reference formulation, whereas this phenomenon was not observed for the test formulation. The compartmental disposition rate constants ($\lambda_1$, $\lambda_z$) and the microconstants ($k_{10}$, $k_{12}$, $k_{21}$; data not shown) were consistent for both fasting administrations, indicating that the disposition of cyclosporine was independent of formulation. Although the doses for the two formulations were chosen to provide comparable cyclosporine exposure under fasting conditions, the test formulation $AUC_b$ was statistically larger than the reference formulation $AUC_b$ ($P < 0.05$).

### Influence of Food on Cyclosporine Pharmacokinetics

When administered with a fat-rich meal, the rate and extent of cyclosporine absorption from the reference formulation were markedly altered. This was manifested by significant prolongations in the lag time and $t_{max}$ and an increase in $AUC_b$. Additionally, the majority of profiles (18/24) exhibited two maxima. As indicated by the point estimates from equivalence testing (Table I), a prolongation in $t_{max}$ by 2.25 hr, without an apparent effect on $C_{max,b}$ but with a 37% increase in $AUC_b$, rendered the rate and extent of cyclosporine absorption from the reference formulation inequivalent under fasting and fed conditions. Additionally, whole-blood cyclosporine concentrations at 12 and 24 hr after administration (corresponding to trough sampling times for a twice-daily and once-daily dosing schedule) were significantly elevated when the reference formulation was administered with food (Table I). The influence of a fat-rich meal on the test formulation was comparatively less. The lag time and duration of apparent zero-order input were unchanged; only three profiles exhibited two input steps. As demonstrated by the point estimates in Table I, $t_{max}$ was prolonged by 15 min, $C_{max,b}$ was reduced by 26% and $AUC_b$ was reduced by 15%. Whole-blood cyclosporine concentrations at 12 and 24 hr after administration were comparable for the test formulation regardless of administration conditions.

## DISCUSSION

A considerable amount of research has been performed to characterize and explain the effect of concomitant food intake on cyclosporine pharmacokinetics. This testifies not only to the clinical importance of this question as regards optimal administration time, patient compliance, and its potential influence on pharmacokinetic variability, but also to the pharmacologic insight it can contribute to elucidate further the mechanisms of cyclosporine absorption and disposition (14). Studies have addressed this issue both in volunteers and in selected transplant patient populations under several different administration conditions (2–6). A general effect of food on cyclosporine pharmacokinetics, which has also been noted for several other drugs (15), is a slowing in the absorption rate. This has been observed at steady state in renal transplant recipients and has been associated with an elevation in the subsequent trough measurements (4,5). It has been suggested that this is one factor which contributes to the variability when trough levels are used in therapeutic drug monitoring (4). More recently, studies have paid par-

**Table I.** Pharmacokinetic Characteristics (Mean ± SD) of Cyclosporine Following Single Oral Administrations of the 300-mg Reference Formulation and 180-mg Test Formulation Under Fasting and Fed Conditions to 24 Healthy Male Volunteers

| | Reference formulation* | | | Test formulation* | | |
|---|---|---|---|---|---|---|
| Characteristic | Fasting | Fed | Equivalence testing[a] | Fasting | Fed | Equivalence testing[a] |
| $t_{lag}$ (hr) | 0.44 ± 0.14 | 1.32 ± 0.72[d] | | 0.42 ± 0.09 | 0.51 ± 0.17 | |
| $t_{lag,2}$ (hr)[b] | 1.72 ± 0.57 | 2.93 ± 1.35 | | — | 1.39 ± 0.91 | |
| $T$ (hr) | 1.49 ± 0.68 | 2.30 ± 1.55 | | 0.96 ± 0.29 | 0.98 ± 0.38 | |
| $T_2$ (hr)[b] | 1.57 ± 0.63 | 2.82 ± 1.38 | | — | 2.52 ± 0.73 | |
| $t_{max}$ (hr) | 2.5 ± 0.9 | 4.8 ± 1.8[d] | 2.25 hr (1.75–3.0 hr) | 1.5 ± 0.4 | 1.8 ± 0.7 | 0.25 hr (−0.25–0.5 hr) |
| $C_{max,b}$ (ng/mL) | 645 ± 248 | 653 ± 266 | 98% (85–118%) | 1011 ± 192 | 759 ± 237[d] | 74% (66–80%) |
| $AUC_b$ (ng · hr/mL) | 3076 ± 1099 | 4174 ± 993[d] | 137% (120–168%) | 3514 ± 878 | 2981 ± 865[c] | 85% (78–91%) |
| $t_{1/2\lambda 1}$ (hr) | 1.2 ± 0.4 | 1.9 ± 0.9[c] | | 1.1 ± 0.2 | 1.3 ± 03 | |
| $t_{1/2}$ (hr) | 7.2 ± 3.5 | 9.5 ± 4.2 | | 7.2 ± 2.8 | 7.7 ± 4.2 | |
| $C(12)_b$ (ng/mL) | 52 ± 19 | 134 ± 68[d] | | 52 ± 17 | 45 ± 17 | |
| $C(24)_b$ (ng/mL) | 14 ± 8 | 27 ± 11[d] | | 15 ± 6 | 12 ± 6 | |

[a] The bioequivalence range for $C_{max,b}$ and $AUC_b$ is 80 to 125%; for reference $t_{max}$ is −0.5 to 0.5 hr and for test $t_{max}$ is −0.3 to 0.3 hr (i.e., ±20% of the respective fasting mean $t_{max}$'s).

[b] Statistics were not performed on these parameters for which the sample sizes were 14 and 18 (reference formulation, fasting and fed) and 0 and 3 (test formulation, fasting and fed).

* Significant within-formulation difference between fasting and fed as assessed by ANOVA: $P < 0.05$ (superscript c); $P < 0.01$ (superscript d).

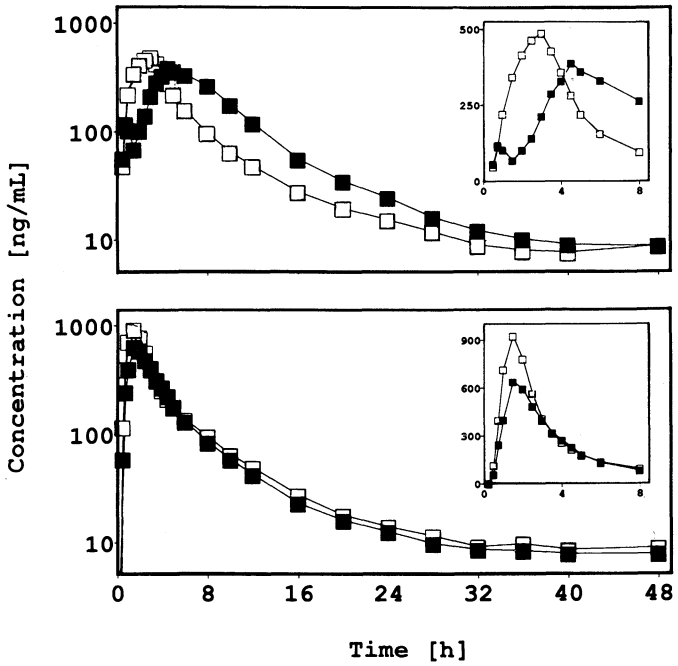


Fig. 1. Geometric mean whole-blood cyclosporine concentration-time profiles following single oral administrations of the 300-mg reference formulation (top) and 180-mg test formulation (bottom) under fasting conditions (□) and with a fat-rich meal (■) to 24 healthy male volunteers.

ticular attention to diet composition (4,6) and appear to concur that a high-fat meal concomitant with a cyclosporine dose is associated with a significant increase in the cyclosporine area under the curve.

The results of the present study for the commercially available formulation confirm both of these food effects. The rate of cyclosporine absorption was slowed in the presence of food as manifested by a delay in the lag time, a nearly doubled time to reach the peak concentration, and a prolongation in the duration of apparent zero-order input. Furthermore, a significant increase in the extent of absorption ($AUC_b$) was noted and was in general agreement with the values previously reported (5,6). The subsequent elevation in cyclosporine concentrations at 12 and 24 hr illustrates the impact which a shift in $t_{max}$ and an increase in $AUC_b$ can produce after only one coadministration of the market formulation with a fat-rich meal. This observation cannot be directly extrapolated to steady-state conditions in patients due to dose nonlinearity and possible long-term pharmacokinetic changes post transplantation (16). However, it does agree well with the significant increases in trough concentrations reported in transplant patients following a single coadministration of a steady-state dose with food (4). The possible impact of this food effect on therapeutic concentration monitoring and subsequent dosage adjustment is recognized in current product labeling which recommends that Sandimmune be administered on a consistent schedule in relation to meals.

In contrast, a fat-rich meal had a less pronounced influence on cyclosporine pharmacokinetics following administration of Sandimmune Neoral. The peak concentration was moderately decreased but the time to reach the maximum was only slightly altered. The extent of absorption ($AUC_b$) showed a small reduction, with the lower confidence limit extending only marginally outside the bioequivalence range. Stability in the subsequent concentration profile was evidenced by comparable blood concentrations at 12 and 24 hr to those obtained under fasting conditions.

The influence of a high-fat meal on cyclosporine bioavailability from the currently marketed formulation is most likely multifactorial (4,14). The results of the present investigation indicate that dosage formulation also plays a role. Indeed, alterations in the formulation can attenuate or minimize the effect of food on cyclosporine absorption. Because both formulations were tested under controlled and identical dietary conditions, differences between the formulations may help to elucidate those factors which make an important contribution to the food effect. Following oral administration of Sandimmune, a crude oil-in-water emulsion is formed in aqueous gastrointestinal fluids, with cyclosporine distributed mainly in lipid droplets. Dispersion of the lipid droplets by bile salts and pancreatin to form mixed micelles is necessary to release cyclosporine for absorption (17). In contrast, Sandimmune Neoral incorporates cyclosporine in a preconcentrate which immediately forms a microemulsion in aqueous fluids simulating a mixed micellar phase. This suggests that circumvention of the lipid dispersion step minimizes the influence of high fat-containing food on cyclosporine absorption and provides support for the hypothesis that a stimulation of bile flow accompanying fat consumption may contribute to the increase in cyclosporine bioavailability from Sandimmune.

In contrast to the food effect observed with the market formulation, a fat- rich meal has a minor influence on the rate

and extent of cyclosporine absorption from Sandimmune Neoral and does not perturb the subsequent blood concentration profile. With Sandimmune Neoral, patients on a fixed cyclosporine dosing regimen have potentially greater freedom with regard to their dietary schedules, which in turn can have a beneficial influence on compliance. Additionally, stability in the concentration profile regardless of food intake is advantageous when trough concentration monitoring is used as feedback for dose titration and in maintaining a stable, individualized dosage regimen.

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