

Improved Dose Linearity of Cyclosporine Pharmacokinetics from a Microemulsion Formulation

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The pharmacokinetic dose proportionality and relative bioavailability of cyclosporine from a microemulsion formulation (Sandimmune Neoral) were compared to those of the commercial formulation (Sandimmune) over the dosage range 200 to 800 mg. Single oral administrations were given as soft gelatin capsules in an open randomized study with 48 healthy volunteers. Whole-blood cyclosporine concentrations were determined by a specific monoclonal radioimmunoassay. In comparison to Sandimmune, the absorption rate (maximum concentration) and systemic availability (area under the curve) of cyclosporine were greater for Sandimmune Neoral at all dose levels investigated. The area under the curve for Sandimmune increased in a less than proportional manner with respect to dose, whereas that for Sandimmune Neoral was consistent with linear pharmacokinetics. Because of this difference, no global assessment of relative bioavailability could be performed. The relative bioavailability of cyclosporine from Sandimmune Neoral ranged from 174 to 239% compared to Sandimmune, depending on the dose level. The improvements in oral bioavailability and dose linearity of cyclosporine exposure after administration as Sandimmune Neoral should facilitate more accurate dosage titration in the clinical setting.

KEY WORDS: cyclosporine; dose proportionality; pharmacokinetics; formulation.

INTRODUCTION

Cyclosporine is a potent immunosuppressive agent which prolongs allograft survival in organ transplantation. In addition, it has also been applied in the treatment of patients with selected autoimmune diseases. Due to its lipophilic nature, currently manufactured formulations of cyclosporine (oral solution and soft gelatin capsules; Sandimmune, Sandoz) employ oil-and-alcohol solutions of the drug (1). The mean absolute bioavailability of cyclosporine from these formulations is approximately 30% but varies markedly as has been demonstrated in several transplant patient populations (2). 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. This investigation was undertaken to assess the dose proportionality and relative bioavailability of the new formulation in comparison to the commercially available formulation in healthy volunteers. The range of doses employed was chosen to represent those in common clinical use in transplantation medicine.

MATERIALS AND METHODS

Subjects

Forty-eight healthy male volunteers aged 31 ± 9 years (mean \pm SD) and weighing 75.2 ± 8.8 kg completed the study. All participants signed a written informed consent after they had been informed of the nature and details of the study. Volunteers were evaluated for general good health on the basis of medical history, physical examination, electrocardiogram, hepatitis A, B, and C, and human immunodeficiency virus (HIV) tests and routine biochemical and hematologic profiles. A screen in urine for drugs of abuse and an alcohol breath test were performed prior to each drug administration; all results were negative.

Study Design

The study protocol was approved by the local medical ethics committee and was performed in accordance with the Declaration of Helsinki and with current European Community and U.S. Food and Drug Administration guidelines for good clinical practice. Because of ethical considerations which limit the exposure of healthy volunteers to cyclosporine, subjects were randomly allocated to one of two parallel study groups within which the order of drug administration was randomized according to a balanced, four-period crossover design. In each group subjects received single oral doses of both study drug formulations at two dose levels, with successive administrations separated by a 2-week wash-out phase. The dose levels were 200 and 600 mg for group A and 400 and 800 mg for group B. The reference cyclosporine formulation was 100 mg soft gelatin capsules of Sandimmune (Sandoz; lot No. Y 144 0890) and the test cyclosporine formulation was 100 mg soft gelatin capsules of Sandimmune Neoral (Sandoz; lot No. X 097 0391). The capsules were ingested together with 250 mL tap water.

Subjects were confined to the study center from 14 hr before until 48 hr after each drug administration. On the days of drug administration, they fasted from 12 hr before dosing until 4 hr after administration. Beginning 4 hr after dosing, subjects were given standardized, scheduled meals which were identical on all dosing days. The time of day of drug intake was identical for a given subject on each dosing occasion. No alcohol was allowed 2 days prior to or during the periods of confinement. Venous blood samples for the determination of cyclosporine in whole blood were obtained pre-dose 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 .

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Bioanalytical Methods

Concentrations of cyclosporine A in whole blood were assayed using the commercially available Sandimmune radioimmunoassay for cyclosporine (Sandoz, Basle), which is based on the use of a monoclonal antibody specific for the parent drug (3). At quality control concentrations of 100, 400, and 800 ng/mL, the respective accuracy was -9.1 , -2.5 , and -2.0% ; the intraassay coefficient of variation was 7.4, 6.0, and 6.8% and the interassay coefficient of variation was 12.7, 11.2, and 6.5%. The overall detection limit calculated from the mean concentration corresponding to 95% binding was 4.6 ± 0.8 ng/mL ($n = 33$). The quantification limit was set to 12.5 ng/mL, the concentration of the lowest sample of the standard curve.

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 quantification and extrapolated to infinity by the addition of the term $\hat{C}(t_z)_b/\lambda_z$, where $\hat{C}(t_z)_b$ and λ_z are the predicted concentration at time t_z and the terminal elimination rate constant determined by log-linear regression analysis. For the regression analysis, weighting of the observed concentrations was inversely proportional to the assay variance. The extrapolations contributed on average 4.2% (range, 0.8 to 16.7%) to the total AUC_b . The terminal disposition half-life was calculated as $t_{1/2} = \ln(2)/\lambda_z$.

Statistical Evaluation

Dose Proportionality. The dose dependency of cyclosporine AUC_b for each formulation was evaluated with the General Linear Model procedure of SAS/STAT (4). Because of the particular design of the study, dose-within-group served as a substitute for the pure dose effect. Any effect of dose-within-group could be practically attributed to dose alone since the standardized environment of the study rendered a group effect unlikely. An additional method previously used to assess the dose proportionality of cyclosporine pharmacokinetics (6) was also applied. Accordingly, the relationship of AUC_b to dose was evaluated by regression analysis through the origin using the procedure FITFUNCTION of the RS/1 software package (5). A linear function [$y = a \cdot x$] and a hyperbolic function [$y = (a \cdot x)/(b + x)$] were fitted to the mean data weighted as $1/(\text{SEM})^2$. The weighted residual sums of squares were subsequently compared by the F test to determine whether the upper order (hyperbolic function) model provided a significant improvement in the fit from the lower order (linear) model (7).

Relative Bioavailability. The relative bioavailability of cyclosporine from the test formulation was estimated from the test/reference AUC_b ratio using the General Linear Model procedure of SAS/STAT. Initially the regression was performed for each dose level separately. Subsequently, a multivariate regression of all dose levels was performed. Fi-

nally, the 95% confidence interval of an overall ratio was computed using Fieller's theorem (8).

RESULTS

Clinical Observations

In general, all four treatments were well tolerated. The most common and probably drug-related adverse event was mild heat sensation of the face, extremities, and epigastric area. The incidence increased in a dose-dependent fashion and, at a given dose level, was higher for the test formulation. The higher incidence was most probably due to the higher blood concentrations of cyclosporine associated with the test formulation. No changes in vital signs, hemograms, urinalysis, biochemistry profiles, or electrocardiograms occurred.

Descriptive Comparison of Formulations

The pharmacokinetic characteristics of cyclosporine are summarized in Table I. As indicated by earlier t_{\max} 's and approximately doubled $C_{\max,b}$'s, cyclosporine absorption was faster from the test formulation irrespective of dose level. The existence of double peaks in several concentration-time profiles confounded a dose-proportionality assessment of $C_{\max,b}$ across the dose range. Fewer double peaks occurred following administration of the test formulation, especially at the lower doses: 10 vs 0 (200 mg), 7 vs 1 (400 mg), 10 vs 8 (600 mg), and 12 vs 12 (800 mg) for reference vs test, respectively. The geometric mean of intraindividual test/reference $C_{\max,b}$ ratios were 1.95, 2.08, 2.00, and 2.05 at the 200- through 800-mg dose levels. There was a trend toward slight but statistically significant increases in terminal half-life with increasing doses irrespective of the formulation. This may, however, be attributable to the common methodological problem that at higher doses the assay is more reliable and therefore the terminal phase can be better resolved.

Dose Proportionality of AUC_b

The boxplots in Fig. 1 demonstrate that the extent of cyclosporine absorption from the test formulation was proportional to the dose, whereas the dose-normalized AUC_b for the reference increased less than proportionally with increasing doses. This was confirmed statistically by a significant dose-within-group effect ($P = 0.0001$) and group effect ($P = 0.004$) for the reference formulation. Neither of the effects was significant for the test formulation ($P = 0.083$ and 0.663, respectively).

Figure 2 shows the relationship between dose and mean AUC_b together with the linear and hyperbolic regression lines. For the reference formulation, the linear relationship [$y = 7.64 \cdot x$] was rejected by the F test [$F(1,2) = 229$, $P < 0.001$], which confirmed the visual impression that a hyperbolic function [$y = (11180 \cdot x)/(919 + x)$] describes the data significantly better. For the test formulation, the results of this analysis confirmed those of the ANOVA: No significant improvement was obtained [$F(1,2) = 6.8$; NS] from the higher-order model [$y = (79954 \cdot x)/(4377 + x)$] over the linear model [$y = 16.3 \cdot x$] for describing the relationship between dose and AUC_b for the test formulation.

Table I. Pharmacokinetic Characteristics (Mean \pm SD) of Cyclosporine Following Single Oral Administrations of the Reference and Test Formulations at Four Dose Levels to Healthy Male Volunteers

| Characteristic | Dose level | | | | | | | |
|--|---------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|---------------------|
| | 200 mg | | 400 mg | | 600 mg | | 800 mg | |
| | Reference | Test | Reference | Test | Reference | Test | Reference | Test |
| t_{max} (hr) | 2.1 \pm 0.7 | 1.5 \pm 0.4 | 2.1 \pm 0.9 | 1.4 \pm 0.4 | 2.3 \pm 1.0 | 1.7 \pm 0.6 | 2.4 \pm 1.0 | 2.1 \pm 0.8 |
| $C_{max,b}$ (ng/mL) | 558 \pm 228 | 1026 \pm 218 | 785 \pm 252 | 1558 \pm 286 | 917 \pm 236 | 1813 \pm 400 | 1046 \pm 264 | 2144 \pm 576 |
| $C_{max,b}/dose$ (ng/mL per mg) | 2.79 \pm 1.14 | 5.13 \pm 1.09 | 1.96 \pm 0.63 | 3.90 \pm 0.72 | 1.53 \pm 0.39 | 3.02 \pm 0.67 | 1.31 \pm 0.33 | 2.68 \pm 0.72 |
| AUC_b (ng \cdot hr/mL) | 2013 \pm 614 | 3471 \pm 1029 | 3336 \pm 1130 | 7054 \pm 1522 | 4518 \pm 1223 | 9831 \pm 2369 | 5256 \pm 1580 | 12364 \pm 3145 |
| $AUC_b/dose$ (ng \cdot hr/mL per mg) | 10.07 \pm 3.07 | 17.36 \pm 5.15 | 8.34 \pm 2.83 | 17.64 \pm 3.81 | 7.53 \pm 2.04 | 16.39 \pm 3.95 | 6.57 \pm 1.98 | 15.46 \pm 3.93 |
| $t_{1/2}$ (hr) | 5.8 \pm 2.4 | 7.0 \pm 2.6 | 7.4 \pm 2.4 | 11.0 \pm 2.9 | 7.2 \pm 2.1 | 9.3 \pm 2.5 | 7.8 \pm 2.1 | 10.3 \pm 1.6 |

Relative Bioavailability

Given the nonlinearity in the dose vs AUC_b relationship for the reference formulation, the relative bioavailability was determined by a regression technique separately at each dose level. An attempt to estimate the 95% confidence limits of a common ratio for all four dose levels using Fieller's theorem was impeded by the relatively small number of observations in relation to the variability. The numerical values of the lower (0.2) and upper (8.5) limit of this ratio reflect the uncertainty. Additionally, multivariate regression analysis of all dose levels did not provide significant coefficients (P val-

ues ranged from 0.062 to 0.959). The relative bioavailability of cyclosporine from the test formulation compared to the reference formulation at each dose level is shown in Table II.

CONCLUSIONS

The dose nonlinearity in cyclosporine pharmacokinetics from the currently marketed formulation (Sandimmune), which was previously demonstrated in a healthy volunteer study over the range 350 to 1400 mg (6), was confirmed in the present study for the parameter area under the curve. In contrast, a new microemulsion formulation (Sandimmune Neoral) exhibited dose proportionality in AUC_b over a wide and clinically relevant dosage range. The rate and extent of cyclosporine absorption from Sandimmune Neoral were greater, but due to the nonlinearity in the AUC_b of the reference formulation, no global relative bioavailability factor could be derived. Depending on the dose level, the relative bioavailability of cyclosporine after administration as Sand-

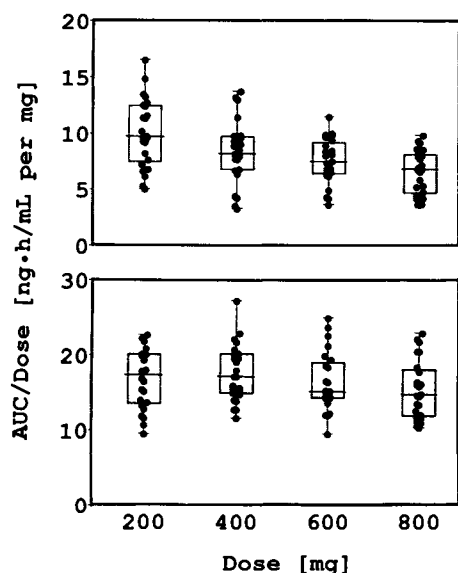


Fig. 1. Boxplot comparing dose-normalized cyclosporine AUC_b at four dose levels following single oral administrations of the reference formulation (top) and the test formulation (bottom) to healthy volunteers. The box, which contains 50% of the observations (\bullet), is divided at the median and has a height equal to the interquartile range. The tails indicate the range of the upper and lower 25% of observations. Not depicted is an outlier value (33.4 ng \cdot hr/mL per mg) for the test formulation at the 200-mg dose level.

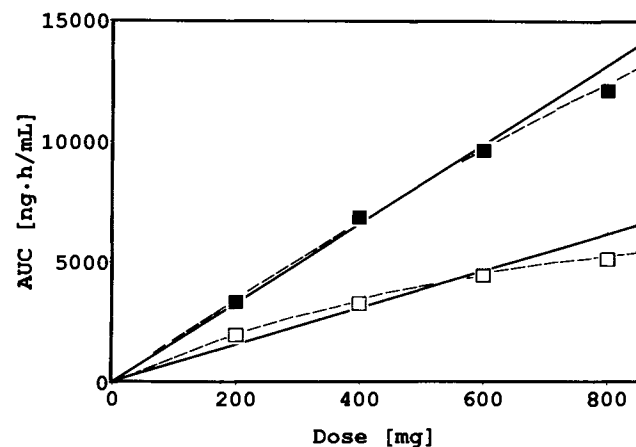


Fig. 2. Relationship between dose and cyclosporine AUC_b following single oral administrations of the reference (\square) and test (\blacksquare) formulations to healthy volunteers. Superimposed is the best fit of a straight line (—) and a hyperbolic function (---) regressed through the origin.

Table II. Relative Bioavailability of Cyclosporine from Soft Gelatin Capsules of the Test Formulation Compared to the Reference Formulation as Assessed in Healthy Volunteers at Four Dose Levels

| Dose level (mg) | AUC _b ratio (test/reference) ^a | 95% confidence interval |
|-----------------|--|-------------------------|
| 200 | 1.74 | 1.58–2.07 |
| 400 | 2.20 | 1.95–2.87 |
| 600 | 2.20 | 1.96–2.71 |
| 800 | 2.39 | 2.12–2.95 |

^a Geometric mean of intraindividual test/reference AUC_b ratios.

immune Neoral ranged from 174 to 239% compared to Sandimmune. The improvements in the oral bioavailability and dose linearity of cyclosporine exposure from Sandimmune Neoral should facilitate more accurate dosage titration in the clinical setting, thereby reducing the risk of over- or underdosing patients.

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