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PHARMACOKINETIC CONVERSION STUDY OF A NEW CYCLOSPORINE FORMULATION IN STABLE ADULT RENAL TRANSPLANT RECIPIENTS

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Cyclosporine A (CyA) is a standard component of immunosuppressive regimens. It is a critical-dose drug for which a minor change in absorption can have important clinical consequences. The aim of the study was to compare the pharmacokinetics and safety of the new generic CyA formulation, Equoral[®] capsules, after a switch from original formulation, Neoral[®] capsules, in seventy stable adult renal transplant recipients. The extent and rate of pharmacokinetic parameters for bioequivalence were compared in a non-randomized, steady-state clinical study with fixed non-replicate study design. Pharmacokinetic analysis of CyA have shown that both the rate and extent of absorption of Equoral[®] does not differ significantly from that of Neoral[®]. At identical dosing, the new formulation was found to have geometric means of C_{\max} 717 ng/ml and AUC_{τ} 3108 ng/ml.h, while corresponding results of comparator were 725 ng/ml and AUC_{τ} 3039 ng/ml.h, respectively. The 90 % confidence intervals of C_{\max} and AUC_{τ} were within 80-125 % interval of the mean values. The results suggest that Equoral[®] capsules can be used as an alternative treatment to Neoral[®] capsules in CyA regimen.

ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the curve
AUMC	Area under the 1 st moment concentration-time curve
BTL	Blood trough level
C ₀ , C ₂	Concentration before and 2 hrs after drug administration
C_{\max}	Maximum concentration
C_{\min}	Minimum concentration
CyA	Cyclosporine A
λ_z	Terminal elimination rate constant
MRT	Mean Residence Time
PK	Pharmacokinetic
PTF	Peak-trough-fluctuation
SD	Standard deviation
ss	steady state
$t_{1/2el}$	Half-life of drug elimination

INTRODUCTION

Cyclosporine, a calcineurine inhibitor, is a potent immunosuppressant with an important role in organ and tissue transplantation. It is a drug with a narrow therapeutic range and therapeutic drug monitoring is important¹.

Pharmacokinetic and pharmacodynamic data suggest that the absorption phase in the first hours after CyA oral administration is an area of the greatest intra- and inter-patient variability. The latter depend upon the patient's individual ability to absorb CyA from the gastrointestinal tract, given possible intestinal diseases, such as mild diarrhea, intestinal surgery or bowel length, and upon subpopulation variability²⁻⁴. Different drug formulations of CyA also represent a very important factor in pharmacokinetic variability. Standard guidelines issued by the Committee for Proprietary Medicinal Products for bioequivalence testing in Europe⁵ consider two medicinal products as bioequivalent if their bioavailability after administration at the same molar dose is similar to such an extent that their efficacy and safety will be essentially the same. Recently, Equoral[®] (IVAX, Miami, Fla, USA), a new formulation of CyA, was tested in a bioequivalence study after single dose administration in healthy volunteers. Equoral[®] proved to be bioequivalent to Neoral[®] (Novartis, Basle, Switzerland) with respect to the extent and the rate of absorption and the drug was marketed⁶. However, standard bioequivalence criteria do not address differences in CyA pharmacokinetics between transplant recipients and healthy volunteers.

The aim of this study was to compare the pharmacokinetics and safety of the new generic CyA formulation - Equoral[®] capsules - after a switch from the original

formulation, Neoral® capsules, in stable adult renal transplant recipients. For this reason, a comparative, non-randomized, steady-state study with fixed non-replicate study design was carried out.

MATERIAL AND METHODS

Subjects

Three centers recruited patients for the investigation after the study protocol had been approved by the local Ethics Committee. Informed written consent was obtained from all study participants. Patient care and the way the study was conducted complied with good clinical practice and the Declaration of Helsinki guidelines.

We included stable renal transplant recipients aged more than 18 years who had undergone renal transplantation of cadaveric or living donors. Patient were included if they had had no rejection episode in the past 6 months, had a stable serum creatinine in the past 3 months with no trend to increase, were normotensive, maintained on CyA in double or triple combination with prednisone, azathioprine, mycophenolate mofetil, had a stable dose of CyA (Sandimmun Neoral® < 8 mg/kg/day, BID) over the previous 14 days prior to entry, had stable concomitant medication 14 days prior to entry. In addition, all patients had three last whole blood CyA trough levels in the range of 70–200 ng/ml. Patients were excluded if they had a severe clinically relevant coexisting disease such as significant cardiac disease or had received a drug which might

influence CyA pharmacokinetics, history of alcohol or drug abuse, malignancy, CMV infection or other unstable medical condition, had received any investigational drugs within the preceding months, were pregnant or lactating, or were tested positively for human immunodeficiency virus.

Design

Study design and schedule of assessments are depicted in Figure 1.

Subjects on stable Sandimmun® Neoral® capsules BID therapy were switched to Equoral® capsules BID at an equivalent dosage (milligrams: milligrams) on day 15 in the morning and then re-switched at an equivalent dosage (milligrams: milligrams) on day 29 in the morning. Individual blood CyA trough levels were checked at entry and on days 18 and 35 to be within therapeutic range 70–200 ng/mL. The sparse sampling, before drug intake, and at 2 hours after drug intake, were measured on day 0 and controlled on day 21. Blood sampling for pharmacokinetic measurement was performed on days 14 and 28. In the afternoon before each 12-hour PK the subjects were admitted and hospitalized in the clinical unit until discharged after the 12-hour pharmacokinetic study. During each pharmacokinetic part of the study 12 venous blood samples were taken; pre-dose and at times 0.5 h; 1 h; 1.5 h; 2 h; 3 h; 4 h; 5 h; 6 h; 8 h; 10 h and 12 h after the regular morning dose of CyA at the end of the first and second study period.

Table 1. Summary of the statistical analysis of multiple-dose pharmacokinetic characteristics

Pharmacokinetic characteristic	Neoral® (R) Geometric mean, n = 12 exp(mean[ln] ± sd[ln])	Equoral® (T) Geometric mean, n = 12 exp(mean[ln] ± sd[ln])	T/R Geometric mean 90%-confidence interval
AUC _τ (ng/ml.h)	3039 (1738, 5314)	3108 (1821, 5304)	1.02 (0.99, 1.06)
C max (ng/ml)	725 (461, 1139)	717 (449, 1147)	0.99 (0.93, 1.05)
C min (ng/ml)	104 (56, 193)	107 (56, 205)	1.03 (0.98, 1.08)
PTF (%)	241 (173, 337)	229 (152, 345)	0.95 (0.90, 1.01)
MRT (h)	8.01 (5.47, 11.77)	8.40 (6.33, 11.15)	1.05 (0.89, 1.21)

Table 2. Number of patients with different absorption profile of cyclosporine

Absorption profile	C ₂ /C ₀	Neoral®	Equoral®
Low	< 3.6	17 (24.3 %)	10 (14.3 %)
Intermediate	3.6–7.6	40 (57.1 %)	46 (65.7 %)
High	> 7.6	13 (18.6 %)	14 (20.0 %)
Total		70 (100 %)	70 (100 %)

$\chi^2 = 2.27$, NS.

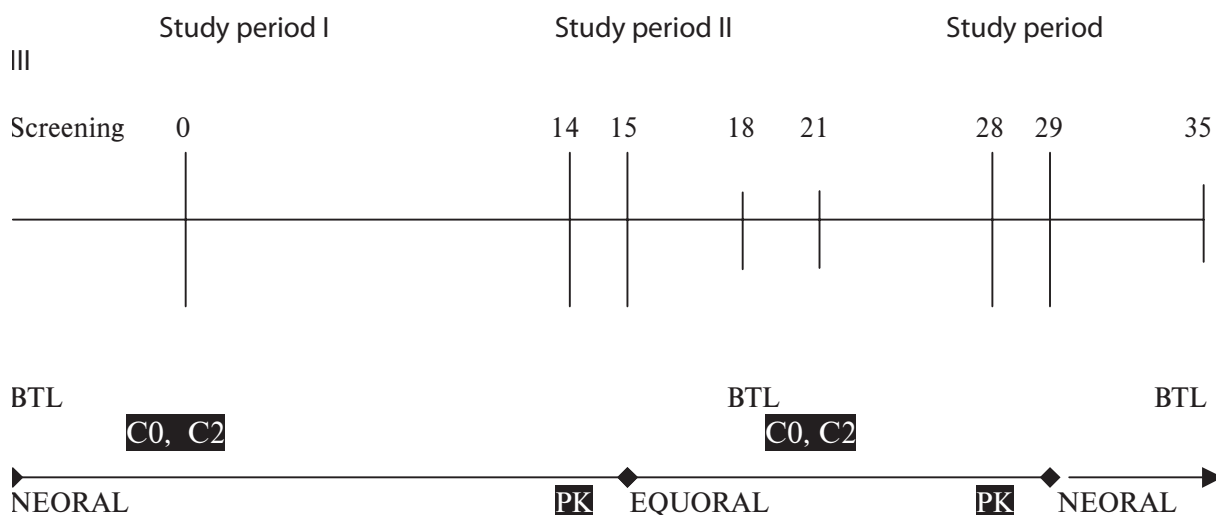


Fig. 1. Study design and schedule of assessments (see abbreviations).

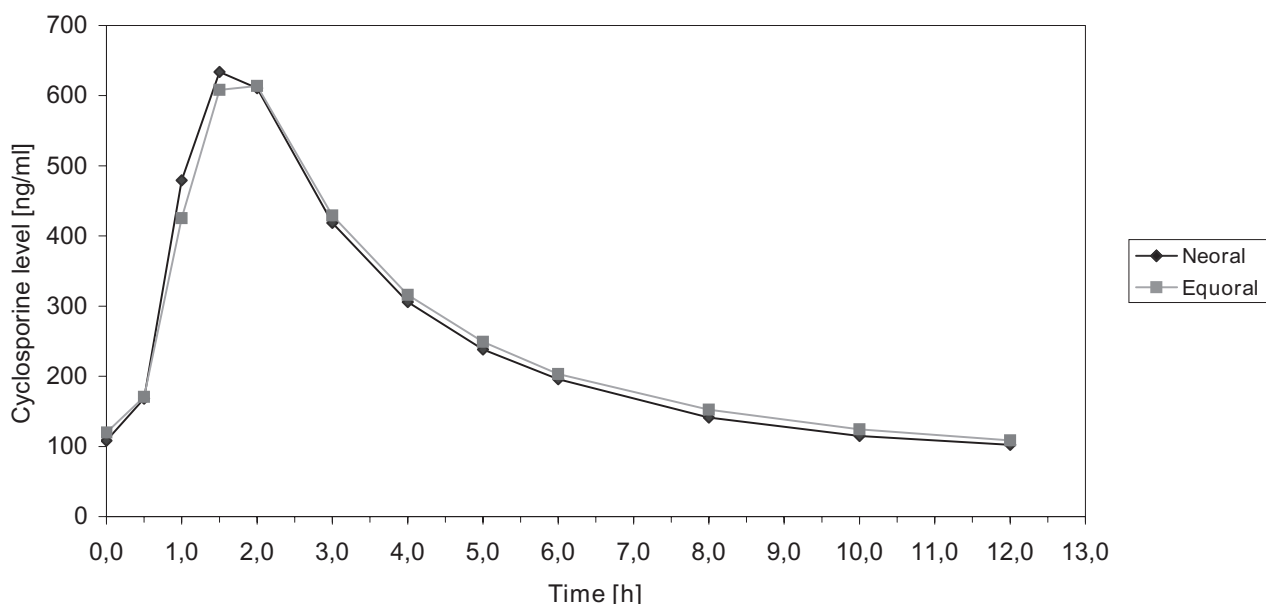


Fig. 2. Geometric means of the concentration/time profiles of cyclosporine.

Safety parameters were monitored at each visit (vital signs, physical examinations, number of routine laboratory parameters, incidence of adverse events).

Analytical Methodology

CyA blood levels were analyzed by a validated TDx Abbot monoclonal specific antibody methodology (limit of quantification - 25 ng/ml) in the local Immunoanalytical Laboratories of each particular center.

Pharmacokinetic and Statistical Analysis

The following extent and rate oriented pharmacokinetic parameters for bioequivalence were individually analyzed by model independent analysis:

- Area under the blood concentration/time curve over one steady-state dosing interval: $AUC\tau$ (determined by the trapezoidal rule up to the last sampling point above the limit of quantification)
- Maximum and minimum blood concentration in the dosing interval C_{max-ss} , C_{min-ss} , (taken directly from the blood concentration/time curve)
- Peak-Trough Fluctuation: $PTF = (C_{max-ss} - C_{min-ss}) / C_{av-ss}$, where $C_{av-ss} = AUC\tau / \tau$
- Terminal elimination rate constant: λ_z (estimated by log-linear least squares regression analysis of the terminal part of the blood concentration/time curve)
- Half-life of drug elimination during terminal phase: $t_{1/2el} = \ln 2 / \lambda_z$
- Mean Residence Time: $MRT = AUMC/AUC$

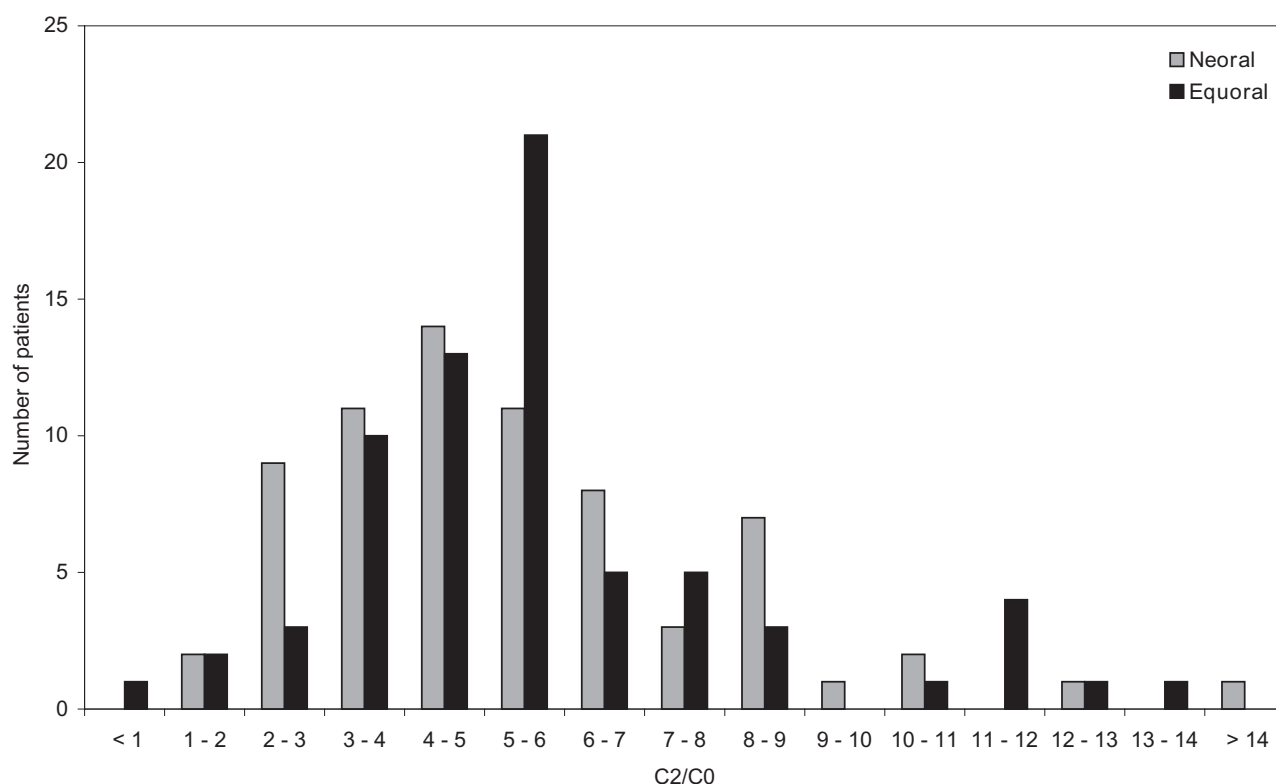


Fig. 3. Distribution of C_2/C_0 values in stable adult renal transplant recipients.

- Relative bioavailability: $F_{rel} = \text{Mean AUC}\tau_{\text{test}} / \text{Mean AUC}\tau_{\text{reference}}$

Computations were performed by means of the KINBES module (version 1.34) of the MW/PHARM software (version 3.30)⁷.

The data were evaluated by geometric mean, standard deviation (SD) and median.

The decision in favour of bioequivalence was based on inclusion of the shortest 90 %-confidence interval for the ratio of medians in the respective bioequivalence range 0.8–1.25.

Significance of the intraindividual differences was tested used a paired t-test and analysis of variance at $p = 0.05$.

RESULTS

Seventy stable adult renal transplant recipients [48 males, 22 females; mean age 35.3males, 34.7 females] were admitted to the study. Average dosage of CyA was 183.9 mg (SD 77.2). Individual patients were maintained on the same dose given as equally divided doses at 12-hr intervals. Figure 2 gives the geometric means of the concentration/time profiles of the reference and test product. The median of maximal concentration after Neoral[®] is 1.5 h with a wide range from 1 to 4 h. Corresponding results for Equoral[®] are 1.5 h with a range from 0.5 to 4 h. Results of the statistical assessment of the rate and extent of absorption after multiple dose of CyA are presented

in Table 1. The results are given as a geometric mean and the shortest 90%-confidence interval for the percent ratio test/reference after logarithmic transformation of the pharmacokinetic characteristics. As can be seen from the Table 1, 90%-confidence interval is entirely in the bioequivalence range of 80 to 125%.

The ANOVA showed that differences in the ratio Neoral[®]/Equoral[®] for individual parameters were not significant among the centers. However, there was a significant difference of C_{min} among centers (Anova; $F = 4.36$, $p < 0.05$).

The mean of $t_{1/2el}$ of the compared formulations were almost identical (Neoral[®] 6.26 h, Equoral[®] 6,31 h). A relatively large inter-individual variability of these elimination characteristics was observed both for Neoral[®] (range 2.13 h –13.96 h) and Equoral[®] (range 3.10–12.12 h). Intraindividual differences were not significantly different (t-test = 0.13).

The geometric mean of the C_0 concentration after the Neoral[®] treatment was 123 ng/ml (range 20 to 447 ng/ml), after the Equoral[®] treatment it was 114 ng/ml (range 13 to 387 ng/ml). The corresponding C_2 were 604 ng/ml (range 122 to 1808 ng/ml) after the Neoral[®] administration and 591 ng/ml (range 85 to 1592 ng/ml) after the Equoral[®] administration. In the 70 stable renal transplant patients, the mean increase of C_0 to C_2 was by factor of 5.4 (SD 2.5) after the Neoral[®] administration, while after the Equoral[®] administration it was 5.7 (SD 2.5). The distribution of the C_2/C_0 levels in these patients is shown in Fig 3. Comparison of number of patients with different absorption profiles is summarized in Table 2. There

was no significant difference in CyA absorption profile of patients after the compared formulations.

The monitored blood trough levels were not significantly changed during the trial (geometric means at screening 116.2 ng/ml, day 18 117.2 ng/ml, and day 35 116.1 ng/ml, Anova; $F=0,02$, $p=0,98$)

CONCLUSION AND DISCUSSION

The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant. However, similarity between the averages in healthy human volunteers does not imply similarity within patients in real clinical usage. Pollard et al formulated a consensus statement regarding potential clinical impact of using different formulations of CyA (ref.⁴). The pharmacokinetic conversion study of the new generic formulation of CyA was performed to exclude the pharmacokinetic problems after conversion from the reference product. We followed the fundamental question: if a patient is allowed to switch between the formulations, how much will the variation in bioavailability increase?

In the clinical study, various pharmacokinetic parameters were used to address the question. Comparison of intrasubject variability of these parameters appears to be a good indicator of switchability⁸.

CyA is a drug with a narrow therapeutic window where monitoring of blood could be of tremendous help¹. Efforts have been directed towards the development of a limited blood sampling strategy for the assessment of the AUC used for therapeutic monitoring⁹. In the past, standard blood trough level (C_0) monitoring has been used. Although this method is currently the routine strategy, after development of the new formulation of CyA, it has become evident that new markers of therapeutic efficacy and toxicity should be tested. The CyA concentration 2 hours after dosing has been shown to be the best single-point predictor of AUC_{0-4} . The C_2 time point has been shown to be more sensitive than C_0 as a surrogate marker for the AUC_{0-4} of Neoral[®] in renal^{10,11}, hepatic¹², and cardiac¹³ transplant recipients.

In our study the C_0 drug level measurement correlated significantly with AUC_{τ} both for Neoral[®] ($r = 0.83$) and Equoral[®] ($r = 0.85$). A significant correlation of AUC_{τ} was also found for C_2 levels (r for Neoral[®] = 0.74, r for Equoral[®] = 0.76). The absorption profile of C_2/C_0 of compared formulation was not significantly different and the data are comparable to the profile described in the literature¹⁴. It is clear that timing of the absorption phase of both formulations is comparable and predictable and therefore both C_0 and C_2 are also applied to the new formulation of CyA.

The pharmacokinetic conversion study of the new CyA formulation Equoral[®] in stable adult renal transplant recipients treated by Neoral[®] showed no differences in the safety parameters monitored.

The study also demonstrated that in the population studied there were no differences in the rate and extent of absorption of the compared formulations, Neoral[®] and Equoral[®]. This is guaranteed to prevent transplant rejection, and it is also an essential prerequisite to avoid overexposure, which places the patient at risk of nephrotoxicity and infection.

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