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Acute effect of cyclosporin on renal function following the initial changeover to a microemulsion formulation in stable kidney transplant patients

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Abstract Potential differences in the acute effect of cyclosporin on renal function when dosed orally as the current market formulation or following a milligram-to-milligram conversion to a new microemulsion formulation were investigated in 14 stable kidney transplant patients. The study consisted of three sequential periods of 2 weeks duration each. Patients entered (period I) and completed (period III) the investigation with the market formulation and received the microemulsion formulation in period II; individualized cyclosporin doses remained unchanged throughout the study. Over one steady-state dosing interval at the end of each study period, whole blood cyclosporin pharmacokinetic profiles were assessed in parallel with endogenous creatinine clearances over sequential 1- to 2-h intervals. The rate and extent of cyclosporin absorption were significantly greater ($P < 0.01$) from the microemulsion formulation with average increases of 73 % in peak concentration and 44 % in area under the curve compared to the market formulation. Sequential creatinine clearances exhibited a transient decrease with the nadir occurring on average between 4 and 6 h post dose followed by a rapid return to baseline. Specifically in period I on the market formulation, clearances decreased from a baseline of 71.7 ± 20.6 to a minimum of 51.1 ± 17.9 ml/min per 1.73 m^2 (similar values in period III) and from 76.8 ± 24.8 to 53.5 ± 17.5 ml/min per 1.73 m^2 in period II on the microemulsion. Neither the baseline nor minimum clearances were significantly different among the study periods. Hence, the pharmacokinetic differences between the formulations did not acutely influence the pattern of glomerular filtration rate following the initial milligram-to-milligram changeover in stable renal transplant patients.

Key words Cyclosporin, renal transplantation, microemulsion · Renal transplantation, cyclosporin, microemulsion · Microemulsion, cyclosporin, renal transplantation

Introduction

A new microemulsion formulation of cyclosporin (Sandimmune Neoral, Sandoz Pharma) has recently been introduced in the clinical management of immunosuppression. The initial changeover from the original oral formulation (Sandimmune) to the microemulsion is generally based on a milligram-to-milligram conversion [5, 9, 12]. While for the majority of stable renal transplant patients trough concentrations remain in the target therapeutic range following the changeover, exposure to cyclosporin (area under the concentration curve; AUC) is increased on average by 30 % and peak concentrations by 60 % due to between-formulation dif-

ferences in cyclosporin absorption [5]. These increases have given rise to concern of a possible adverse influence on renal function. However, 3-month follow-up data in over 450 stable renal transplant patients who have changed over to the microemulsion formulation have not revealed any significant changes in serum creatinine or blood urea nitrogen (BUN) [12].

In recognition of the limitations in using serum creatinine to register possible alterations in glomerular filtration rate (GFR), the present crossover study was undertaken to focus on the early postconversion period with a more specific index of glomerular filtration. To accommodate the study to a clinic setting and allow a sufficient number of patients to be assessed, we opted to perform sequential endogenous creatinine clearance (CL_{cr}) measurements in parallel with pharmacokinetic sampling over a steady-state dosing interval on an outpatient basis. The method chosen could be easily applied in this setting while being sufficiently sensitive to reproducibly detect the transient decrease in GFR and its return to baseline, which has been previously described in renal transplant patients receiving Sandimmune [10].

Materials and methods

Study design

The protocol was approved by a local medical ethics committee and the study 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. All subjects gave written informed consent for participation in the study. The 6-week investigation consisted of three sequential periods. In period I (study weeks 1–2), stable renal transplant patients were enrolled and continued to receive their individualized dose of the reference formulation (Sandimmune soft gelatin capsules) with the total daily dose divided into two equal doses administered every 12 h. In period II (study weeks 3–4), patients were changed over to the microemulsion formulation (Sandimmune Neoral soft gelatine capsules) at the same dose as in period I. Based on trough concentration monitoring, the dose could subsequently be adjusted if necessary. In period III (study weeks 5–6) the reference formulation was reinstituted at the same dose as at study initiation.

Interventions

Tolerability and safety were monitored at clinic visits during each study period and at study completion. At these visits weight, blood pressure, pulse rate, routine laboratory chemistries/urinalysis and fasting total cholesterol, total triglycerides and glucose were assessed. A 12-lead ECG was performed at study entry and completion. A morning predose cyclosporin concentration was assessed weekly throughout the study.

Pharmacokinetic and CL_{cr} assessments were performed over a morning dosing interval within the last 3 days of each study period. On these days patients reported to the study center after an overnight fast. They received their morning dose followed immediately by a continental breakfast. Because of a possible influence of circadian rhythm on GFR [3], the administration time for the morning dose was identical for each subject on all three assessment occasions. Additional standardized meals were served at 4, 8, and 10 h after the dose administration. Venous blood samples for the determination of cyclosporin in whole blood were obtained predose and then 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, and 12 h thereafter. Samples were collected in EDTA-containing tubes, gently inverted several times, and frozen at –20 °C. GFR was assessed by sequential endogenous creatinine clearances. Subjects were instructed to ingest 300 ml tap water upon awakening and an additional 10 ml/kg were given upon arrival at the clinic. A continuous, intravenous 250-ml/h infusion of isotonic saline solution was started at least 30 min before the first urine collection and was maintained over the 12-h dosing interval with rate adjustments as necessary to maintain a roughly constant urine flow. Urine samples were collected by spontaneous voiding over nine collection intervals: 0–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–10, and 10–12 h after dose administration. Blood samples for serum creatinine determinations were taken before dose administration and every 2 h over the dosing interval. Creatinine in serum and urine was analyzed via a validated, automated, enzymatic colorimetric test.

Bioanalytical methods

Concentrations of cyclosporin in whole blood were assayed using the commercially available radioimmunoassay kit (Sandoz, Basel, Switzerland), which is based on the use of a monoclonal antibody specific for the parent compound [1]. At quality control concentrations of 6.25, 12.5, 100, 400, and 1600 ng/ml, the respective accuracies were –3.0 %, 0.4 %, –12.3 %, –6.4 %, 1.4 %; the intra-assay coefficients of variation were 32.8 %, 17.7 %, 5.2 %, 3.0 %, and 6.8 % and the interassay coefficients of variation were 14.5 %, 6.9 %, 4.1 %, 5.8 %, and 5.8 %. The overall detection limit calculated from the mean ± SD concentration corresponding to 95 % binding was 5.0 ± 0.6 ng/ml ($n = 9$). The quantification limit (intra-assay coefficient of variation ≤ 30 %) was set at 12.5 ng/ml.

Pharmacokinetic and CL_{cr} evaluations

Whole blood steady-state cyclosporin concentration-time data were analyzed by standard noncompartmental methods. The highest measured blood (b) concentration and the corresponding sampling time were defined as $C_{max,b}^{ss}$ and t_{max}, respectively. The measured blood concentration 12 h after the profiled dose was defined as $C_{min,b}^{ss}$. The $AUC_{\tau,b}^{ss}$ was calculated from 0 h to 12 h using the trapezoidal rule. The average steady-state concentration $C_{av,b}^{ss}$ was calculated as $AUC_{\tau,b}^{ss}/\tau$, where τ is the dosing interval, and the percent peak-trough fluctuation (%PTF) was calculated as $[(C_{max,b}^{ss}-C_{min,b}^{ss})/C_{av,b}^{ss}] \cdot 100$. CL_{cr} for each sampling interval was calculated as the product of urine creatinine concentration and urine volume divided by the serum creatinine concentration. CL_{cr} was normalized to 1.73 m^2 body surface area [4]. The minimal clearance over the dosing interval was designated as CL_{min}. Because the study was conducted at steady state, the baseline clearance ($CL_{baseline}$) was chosen as the clearance from the 10–12 h interval since, at this time, cyclosporin concentrations are reaching trough levels with little change over this 2-h interval.

Statistical evaluation

Pharmacokinetic parameters and clearances were compared by ANOVA with subjects and treatments as sources of variation after

Table 1 Steady-state cyclosporin pharmacokinetic parameters and creatinine clearances (mean ± SD) in stable renal transplant patients receiving individualized doses of the reference and the microemulsion formulations

Parameter	Reference formulation (Period I)	Microemulsion formulation (Period II)	Reference formulation (Period III)	Statistical comparison[a]		
				I vs II	I vs III	Power
Dose (mg)	114 ± 23	114 ± 23	114 ± 23	–[b]	–[b]	
$C_{min,b}^{ss}$ (ng/ml)	73 ± 21	90 ± 29	73 ± 18	$P < 0.01$	NS	0.84
t_{max} (h)	1.9 ± 1.3	1.1 ± 0.2	2.4 ± 1.5	$P < 0.05$	NS	0.13
$C_{max,b}^{ss}$ (ng/ml)	545 ± 257	872 ± 214	546 ± 177	$P < 0.01$	NS	0.19
$AUC_{\tau,b}^{ss}$ (ng · h/ml)	2128 ± 527	3023 ± 539	2235 ± 453	$P < 0.01$	NS	0.70
$C_{av,b}^{ss}$ (ng/ml)	177 ± 44	252 ± 45	186 ± 38	$P < 0.01$	NS	0.70
PTF (%)	262 ± 115	313 ± 69	253 ± 67	NS	NS	0.31
$CL_{baseline}$ (ml/min per 1.73 m^2)	71.7 ± 20.6	76.8 ± 24.8	71.3 ± 22.9	NS	NS	0.65
CL_{min} (ml/min per 1.73 m^2)	51.1 ± 17.9	53.5 ± 17.5	51.7 ± 16.2	NS	NS	0.84

[a] Results of ANOVA; power to detect a ± 20 % difference from the period I mean
[b] Doses unchanged throughout the study

ascertaining normality of distribution (Wilk-Shapiro statistic) and homogeneity of variances (Levene test). Individual treatments were subsequently compared with the Newman-Keuls multiple comparisons test. The overall magnitude of the change in a pharmacokinetic parameter when comparing the test and reference formulations was expressed as a percentage increase or decrease based on the ratio of the geometric means. All statistical hypotheses were tested at the 0.05 level of significance. Data are represented as mean ± SD.

Results

Study population

Fourteen patients (five women, nine men) completed the study. They were 46.5 ± 9.4 years of age (range 26–59 years) and weighed 72.3 ± 12.4 kg (range 53–92 kg). At study entry, the time post-transplant ranged from 10 to 103 months. In addition to cyclosporin, immunosuppressive regimens included steroids for 12 patients and steroids with azathioprine for 2 patients. Antihypertensive therapy included calcium channel blockers in 9 of the 14 subjects. These and other concomitant medications and doses were kept constant over the study duration. None of the patients received comedications known to interact pharmacokinetically with cyclosporin.

Safety and tolerability

Both study drug formulations were well tolerated. Two patients had urinary tract infections (UTI) that responded to appropriate therapy; both patients had histories of recurrent UTI. Hemograms, biochemistry profiles, and urinalysis assessments remained stable in all patients throughout the study. Proteinuria was absent or minimal in all subjects with one exception (1.7 g/day); there was no change in these data throughout the study.

Pharmacokinetics of cyclosporin

Cyclosporin doses and pharmacokinetic parameters are compiled in Table 1; the mean concentration profiles from periods I and II are depicted in Figs. 1 and 2, respectively. Doses remained unchanged throughout the study, ranging from 75 to 150 mg/dose (0.8–2.9 mg/kg per dose). Following the milligram-to-milligram conversion to the microemulsion formulation in period II, $C_{min,b}^{ss}$ was significantly elevated; however, concentrations remained in the target therapeutic range and did not prompt dose adjustments. The rate of cyclosporin absorption was faster from the microemulsion as evidenced by an earlier t_{max} and an average increase of 73 % in $C_{max,b}^{ss}$. Absorption occurred more consistently in a single absorption step for the microemulsion formulation (one profile with a double peak) than for the reference formulation (five and six profiles with double peaks in periods I and III, respectively). The extent of absorption ($AUC_{\tau,b}^{ss}$) was, on average, 44 % greater from the microemulsion formulation. Upon reinstitution of the reference formulation in period III, pharmacokinetic parameters were similar to those at study entry.

Sequential creatinine clearances

The average urine flow ranged from 4.5 to 11 ml/min. The urinary creatinine excretion rate (the product of urine creatinine concentration and urine volume) was independent of serum creatinine and of urine flow. Serum creatinine exhibited negligible fluctuations over the dosing interval. There were no significant differences in $CL_{baseline}$ across the study periods; likewise CL_{min}, which occurred on average between 4 and 6 h after dose administration was comparable regardless of the drug formulation as shown in Table 1 and Figs. 1 and 2. The average acute decrease in clearance was

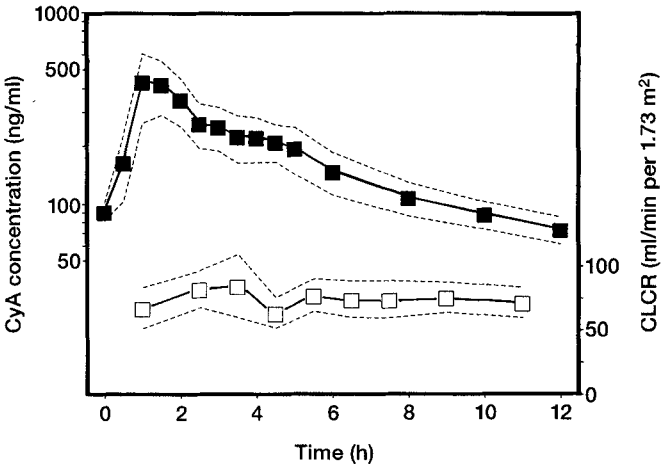

Fig. 1 Mean (——) with 95 % confidence interval (- - - -) profiles of cyclosporin whole blood concentrations (■) and sequential endogenous creatinine clearances (□) during a steady-state dosing interval in patients receiving the reference formulation in period I. Creatinine clearance values are plotted at the midpoint of the collection interval

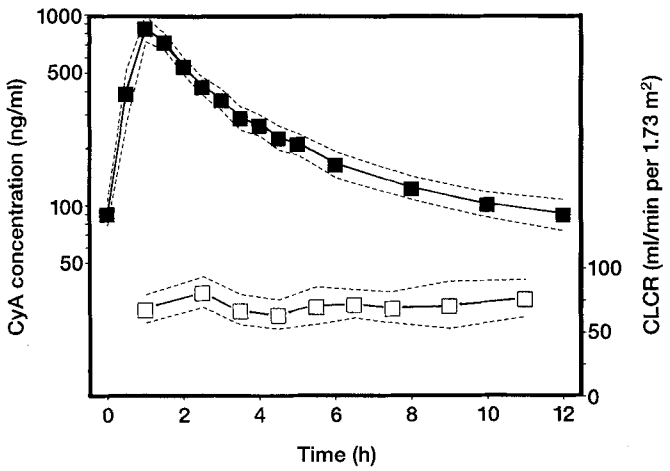

Fig. 2 Mean cyclosporin whole blood concentrations and sequential creatinine clearance profiles in patients receiving the microemulsion formulation in period II. Symbols as in Fig. 1

30 % from baseline in all study periods. There was no correlation between the percent decrease in CL_{cr} from baseline and cyclosporin $AUC_{\tau,b}^{ss}$ ($r = 0.018$, $P = 0.91$) or $C_{max,b}^{ss}$ ($r = 0.048$, $P = 0.76$). The overall pattern of a transient decline in CL_{cr} and its return to baseline over the dosing interval was generally more consistent between patients during treatment with the microemulsion formulation than with the reference formulation, which may reflect the lower pharmacokinetic variability from the microemulsion.

Discussion

A new microemulsion formulation of cyclosporin (Sandimmune Neoral) has been extensively tested in stable renal transplant patients and demonstrates a more consistent and predictable pharmacokinetic profile than the currently marketed formulation (Sandimmune) [5, 9, 12]. The initial changeover in stable patients is generally based on a milligram-to-milligram conversion [5, 9, 12]. Due to absorption-related differences in the two formulations, higher exposure to cyclosporin occurs on average following the changeover. The aim of the present investigation was to survey and compare the relationship between cyclosporin pharmacokinetics and GFR in stable renal transplant outpatients receiving both oral formulations in a crossover protocol.

To accommodate the survey to a clinic setting, GFR was determined by the sequential endogenous creatinine clearance method. This method shows a good correlation with other GFR assessment methods, such as polyfructosan clearance [11] and inulin clearance [8], in clinically stable renal transplant patients. The overestimation of GFR using endogenous creatinine clearance due to additional tubular secretion is below 15 % when GFR is greater than 30 ml/min [2, 7], which is usually the case in this population. Additionally in this investigation, experimental conditions for the assessment of GFR were validated by several measures: creatinine excretion rates were independent of serum creatinine concentration and urine flow, urine flow was sufficient to meet the methodological requirements of induced water diuresis [6], and serum creatinine concentrations exhibited only negligible fluctuations during the sampling periods. Finally, the method was sensitive enough to detect the transient decrease in GFR previously reported in patients receiving the market formulation [10] while the consistency in results between periods I and III (both with the market formulation) demonstrate the reproducibility of the measurements. With regard to the pharmacokinetic assessments, the resulting increase in cyclosporin exposure following the milligram-to-milligram changeover in the present study is in agreement with that previously reported [5], thereby confirming the relevance of the pharmacokinetic results as representative for this patient population.

Sufficient statistical power was achieved to demonstrate that the minimum clearance during the dosing interval was not influenced by the formulation. The transient decline that occurred on average between 4 and 6 h after dosing was quickly reversible in all study periods. This GFR pattern is similar to that reported by Perico et al., who studied the effect of cyclosporin on inulin clearance and renal perfusion in seven renal transplant patients [10]. Following single oral doses of the market formulation at four dose levels, a transient, rapidly-reversible renal hypoperfusion and a decrease in

GFR occurred 2–4 h after the peak drug concentration. The authors noted a dose-dependency in GFR reduction with a 63 % reduction at single doses of 5 mg/kg, 35 % at 2.5 mg/kg and 18 % at 1.5 mg/kg. Over this dosage range the group mean $AUC(0–12)_b$ values ranged from 1919 to 5462 ng · h/ml and $C_{max,b}$-values from 493 to 1205 ng/ml. In the present study in stable renal transplant patients at steady state, doses ranged from 0.8 to 2.9 mg/kg with an average 30 % acute decrease in GFR. There was no evidence that the extent of GFR reduction was dependent on cyclosporin exposure. This is most likely attributable to the fact that even with the increase in cyclosporin bioavailability from the microemulsion formulation, $AUC_{\tau,b}^{ss}$ values (group means 2128–3023 ng · h/ml) and $C_{max,b}^{ss}$ values (group means 545–872 ng/ml) span a relatively smaller range in association with the narrower dose range used in stable patients at steady state.

In summary, these acute data compliment the results reported in a large number of stable renal transplant patients that indicated stability in serum creatinine over the first 3 months following conversion to the microemulsion formulation [12]. Additionally, they provide some reassurance that the higher exposure to cyclosporin following the initial milligram-to-milligram conversion from the market to the microemulsion formulation does not acutely influence GFR in stable renal transplant patients. Clearly, the results of this survey need to be followed up by longitudinal assessments of renal function in this population.

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