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New cyclosporine microemulsion randomized, cross-over bioequivalence steady-state study in renal transplanted patients

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Abstract. – A new microemulsion formulation of cyclosporine was compared with the marketed formulation in 18 stable renal transplanted patients.

Aim of the study was not only to determine the bioequivalence between the two pharmaceutical preparations, but also to ascertain whether tested drug could maintain stable blood concentrations of cyclosporine.

Renal transplanted patients under cyclosporine treatment from at least 12 months at a well individualized dosage (resulting in 90-200 ng/mL of blood level drug) have been selected.

Patients received the same preceding dose of cyclosporine through both the two preparations according to a cross-over, randomized schedule during 4 weeks in two equally divided daily administrations. Serial blood samples were obtained over a 24-hour period at steady-state of each formulation. Cyclosporine concentrations were determined by a specific immunoassay method (FPIA) n whole blood taken in the last day of each cycle of treatment. Statistical comparisons of cyclosporine levels (using pharmacokinetic parameters) were cross-performed between formulations and days of blood test.

Tested drug resulted bioequivalent with the reference marketed formulation. Furthermore, the study showed that tested drug maintained satisfactory stable blood concentrations of cyclosporine.

Key Words:

Cyclosporine A, Bioequivalence, Microemulsion, Pharmacokinetics, Immunosupression, Renal transplant.

Introduction

Cyclosporine A is a well established immunosuppressant agent that dramatically improves the survival after organ transplantations[1-3].

However, its absolute bioavailability and pharmacokinetics are highly variable within among patients and generally unpredictable thus requesting frequent monitoring determinations. Different factors contribute to this behaviour of cyclosporine: high molecular weight, poor solubility in water and in aqueous fluid, high lipophilicity, and food composition of fed patients[4]. Up to a few years ago, but not infrequently today as well, the common pharmaceutical preparations were oil based solutions in bottles or in soft gelatine capsules.

Recently, in order to optimise cyclosporine oral absorption, new formulations have been developed that incorporate the drug in preconcentrate. These pharmaceutical forms (known as "microemulsions") have been proved to produce a better dose-effect relationship[5].

Microemulsions are characterized by the capacity to form microsuspension in aqueous and gastric fluids thus stimulating a mixed micellar phase. The result is a highly improved absorption of cyclosporine along with the entire gastrointestinal tract[5-7].

Sigmasporin Microral is a new microemulsion made by mixing the drug with lipophilic and hydrophilic solvents coupled with a suitable surfactant. The pattern of particle sizes in the microemulsion, checked with laser light scattering method[8], results highly uniform.

The aim of the present study was to evaluate the pharmacokinetic profile and safety of a new cyclosporine A formulation* in renal transplanted patients comparing it with a recently marketed microemulsion∞.

Materials and Methods

Both drugs used[*-∞] contained cyclosporine A at the concentration of 100 mg/mL.

Eighteen stable transplanted adult, caucasian recipients (10 male, 8 female; age ranged 26-58 years) were enrolled. The criteria of selection were successful transplantation and a period of at least 12 months from transplantation.

Before acceptance for the experiment, all patients passed a medical examination to verify:

- no evidence of acute graft rejection;
- stable renal graft function defined as serum creatinine up to 2 mg/L above the patient established baseline;
- the compliance to a double (corticosteroids + cyclosporine) or triple (corticosteroids + azothioprine + cyclosporine) immunosuppressive therapy;
- oral administration of constant, personalized amount of cyclosporine A microemulsion in two daily doses for at least 8 weeks prior the study;
- regular weekly controls of cyclosporine blood levels for at least 8 weeks prior the study;
- no excessive variations in preceding cyclosporine levels exceeding 90-200 ng/mL;
- biochemical and hematological values within the usual range.

Exclusion criteria were:

- patients assuming drugs interfering with absorption, metabolism and toxicity of cyclosporine A (for instance, macrolide antibiotics, ketoconazole, β-adrenergic blockers, Ca-antagonist, anticonvulsivants);
- concomitant pathology such as severe diabetes, liver diseases, hypertension, gastrointestinal disturbances or any other disorder interfering with the pharmacokinetic process;
- alcohol and cigarette consumption;
- positivity to Hepatitis B, C and HIV test.

The study was conducted in accordance with the principles laid down in the declarations of Helsinki, Tokyo, and Venice concerning biomedical research involving human subjects. In particular the study protocol was subject to the institutional ethical review board's approval. Written informed consent was obtained from every subject prior to his/her inclusion in the study.

All patients have been treated during the 8 weeks before the study with a constant and well individualized dose of a marketed cyclosporine A microemulsion: 4-5 mg/kg, divided in two equal parts during each day, at 8:00 and 20:00.

The study was divided into two sequential periods of 4 week duration each.

According to a randomisation (computerized) schedule, the subjects received test formulation or reference formulation in the first period of 4 weeks. In the second period (4 weeks duration) the sequence of drug administrations were reversed.

Both drugs were taken orally with 150 mL of tap water (or other beverage as home habits) at the same daily dose of cyclosporine with which they were enrolled in the study. The total daily dose was divided in two equal parts and administered at 08:00 and at 20:00 hrs. Breakfast, lunch and supper were provided at 10:00, 13:00, and 19:30, respectively. No concomitant medication was administered during the whole study except corticosteroids and azothioprine.

Tolerability and safety were monitored just before and during the entire experiment period.

Physical examination, blood pressure measurements, and laboratory tests were carried out at enrolment and were repeated periodically.

Following 4 weeks of treatment with Test preparation or Reference preparation, the blood cyclosporine concentration was determined at the following times: at predose (0) and 15 min, 30 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 13, 14, 16, 20, 24 hours after the morning dose.

Concentration of cyclosporine in the whole blood was measured by Fluorescence Polarization Immunoassay (FPIA) technology.

Pharmacokinetic Parameters and Statistical Evaluations

The following pharmacokinetic parameters were measured according to the partition of the blood cyclosporine content measurements, into two time phases: "1st Phase", from 0 to 12:00 o'clock, "2nd Phase", from 12:00 to 24:00 o'clock.

[*] SIGMASPORIN MICROORAL – Sigma Pharma Sao Paulo, Brazil.

[∞] SANDIMMUN NEORAL® – Sandoz-Wander Pharma SA, Switzerland.

- AUCx-y was calculated by the trapezoid rule ("x" was 0 or 12, and "y" was 12 or 24, depending on the "phase");
- AUC_{t-z} calculated according to the formula: $AUC_{t-z} + Ct/\beta$. Ct was the last concentration estimated and β = slope of the phase of elimination; "t" was 0 or 12, and "y" was 12, or 24, or depending on the "phase".
- C_{max} and t_{max} were the observed values.

The statistical evaluations were performed as follows:

- in the "1st Phase" the pharmacokinetic parameters of "0 time" were compared with those measured at 12 hours after the drug;
- in the "2nd Phase", the comparisons were between performed the mean values of the 12th hour and those of the 24th hour.

Analysis of variance was on the pharmacokinetic parameters AUC_s and C_{max} after logarithmic transformation, using the *General Linear Model* procedure. Factors accounting for the sources of variation were: sequence, subjects (in sequence), period and treatment. The 90% confidential intervals for the ratio between the test and reference average of AUC_s and C_{max} were calculated according to Westlake[9] for which bioequivalence should lie within the ratio 0.8-1.25 for AUC_s and 0.7-1.43 for C_{max}.

A "p" value less than 0.05 was adopted as the criterion for the significance of observed differences.

The parameter t_{max} were evaluated by a non-parametric procedure, according to the Wilcoxon matched-pairs rank test.

The comparisons among the time intervals 0-12th hour, 1-13th hour, and 2-14th hours were performed using the logarithms of blood level concentrations. ANOVA was established according to cross-over model.

The comparison of pharmacokinetic parameters between the "1st Phase" (0-12th h) and the "2nd Phase" (12th-24th h) has been performed using the difference (Δ) of the AUC_s and C_{max} logarithms. Using these parameters, ANOVA test was calculated according to the cross-over model and the ANOVA cross-over model for repeated measurements in which the times (or "Phases") were also taken into account.

Results

A. Results from the "1st Phase" (the 0-12 hours time period after the morning treatment of the two preparations).

The concentrations of cyclosporine in the whole blood after treatment with Test preparation and Reference preparation during the first 12 hours (1st Phase) are reported in Table I: the drugs concentration increased gradually up to one hour and half (time of the maximal blood concentration) and decreased slowly reaching values between 117 and 120 ng/mL.

Table II reports the AUCs, the Cmax and the tmax as mean ± SD calculated during the 1st Phase. There were no statistical differences among each couple of means (Tables III and IV).

Table I. Concentration of cyclosporine in the whole blood after treatment with Test preparation and Reference preparation: 0-12 hours (mean values in ng/mL ± SD).

Hours after morning treatment	Test preparation	Reference preparation
0	122.17 ± 28.03	126.00 ± 31.64
15 min	224.75 ± 78.33	214.67 ± 70.84
30 min	425.95 ± 163.49	397.23 ± 134.25
1	732.66 ± 245.26	718.06 ± 239.78
1.30	983.76 ± 212.22	1008.41 ± 214.93
2	904.65 ± 220.78	845.48 ± 205.51
3	512.19 ± 107.71	465.64 ± 83.91
4	405.10 ± 91.47	396.16 ± 97.08
6	272.98 ± 48.12	298.86 ± 65.01
8	205.99 ± 60.85	197.79 ± 69.07
10	144.63 ± 48.37	150.59 ± 51.34
12	117.20 ± 27.87	120.21 ± 34.83

Table II. Mean values (± SD) of pharmacokinetic parameters.

Treatments	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}	t_{max}
Test preparation	4318.107 ± 653.847	4907.499 ± 804.880	1103.578 ± 133.591	1.556 ± 0.338
Reference preparation	4254.204 ± 704.586	4886.257 ± 903.304	1104.907 ± 108.032	1.528 ± 0.320

Table III. Statistical evaluation - P values of the ANOVA.

p-value	Log AUC_{0-t}	Log $AUC_{0-\infty}$	Log C_{max}
Sequence	0.974	0.931	0.085
Treatments	0.334	0.618	0.882
Periods	0.818	0.799	0.833

Table V reports the averages of treatments and the 90% confidence limits for the above analysed variables. The 90% confidential interval for AUCs-ratio and C_{max}-ratio lays within the bioequivalence range of 0.80-1.25 and 0.7-1.43 respectively.

The value of variables calculated according to Westlake are within the confidential limits of 20-25%, thus indicating the bioequivalence of the two preparations.

B. Results from the "2nd Phase" (the 12-24 hours time period after the morning treatment of the two preparations).

Like the Section A), the mean values (± SD) of cyclosporine concentrations in the whole blood after treatment with Test preparation and Reference preparation after the 12- hours "2nd Phase" are reported in Table VI.

The time course of the blood drug concentrations of both preparations is depicted in Figure 1. The maximum level of both drugs was reached on the 14th hour.

Table VII reports the mean values (± SD) of AUC_{12-24}, $AUC_{12-\infty}$, C_{max}, and t_{max}; no statistical differences among each couple of means have been noted, t_{max}, resulted not statistically significant by the Wilcoxon test (p = 0,317) (Tables VIII and IX).

Table X reports the averages of treatments and the 90% confidential limits for the above analysed variables. The 90% confidential interval for AUC-ratio and C_{max}-ratio lays within the bioequivalence range of 0.80-1.25 and 0.7-1.43 respectively.

The values of variables calculated according to Westlake are within the confidential limits of 20-25%, thus indicating the bioequivalence of the two preparations.

Table IV. Statistical evaluation – p value of the Wilcoxon test.

	p-value
t max	0.593 ns

Table V. Statistical evaluation - mean values of pharmacokinetics parameters and CI 90% according to Westlake.

	Log AUC_{0-t}	Log $AUC_{0-\infty}$	Log C_{max}
Test preparation	3.630	3.685	3.040
Reference preparation	3.623	3.682	3.04
Lower limit	0.987	0.980	0.953
Test preparation/Reference preparation	101.812	100.856	99.609
Upper limit	1.051	1.038	1.041

Table VI. Concentration of cyclosporine in the whole blood after treatment with Test preparation and Reference preparation: 12-24 hours (mean values in ng/mL ± SD).

Hours after morning treatment	Test preparation	Reference preparation
12	117.20 ± 27.87	120.21 ± 34.83
13	674.75 ± 230.48	651.22 ± 237.42
14	848.60 ± 149.94	821.47 ± 196.89
16	449.29 ± 129.48	412.28 ± 108.35
20	185.88 ± 44.00	194.73 ± 43.51
24	133.50 ± 33.26	137.32 ± 34.42

C. Statistical comparisons "among times" of blood cyclosporine levels after Test preparation and Reference preparation administrations.

Table XI reports the p values calculated, by the ANOVA test, on the differences (Δ, expressed as logarithms) of cyclosporine blood levels at the following times: 0 vs. 12th hour, 1st vs. 13th hour, and 2nd vs. the 14th hour. No significant difference has been detected.

Table XII reports the averages of treatments and the 90% confidential limits for the above analysed variables. The 90% confidential interval for AUC-ratio and C_{max}-ratio lays within the bioequivalence range of 0.80-1.25 and 0.7-1.43 respectively. The values of variables calculated according to Westlake are within the confidential limits of 20-25%, thus indicating the bioequivalence of the two preparations.

D. Statistical comparisons "between phases" of the pharmacokinetic parameters.

Table XIII reports the p values calculated, by the ANOVA test, on the differences (Δ,

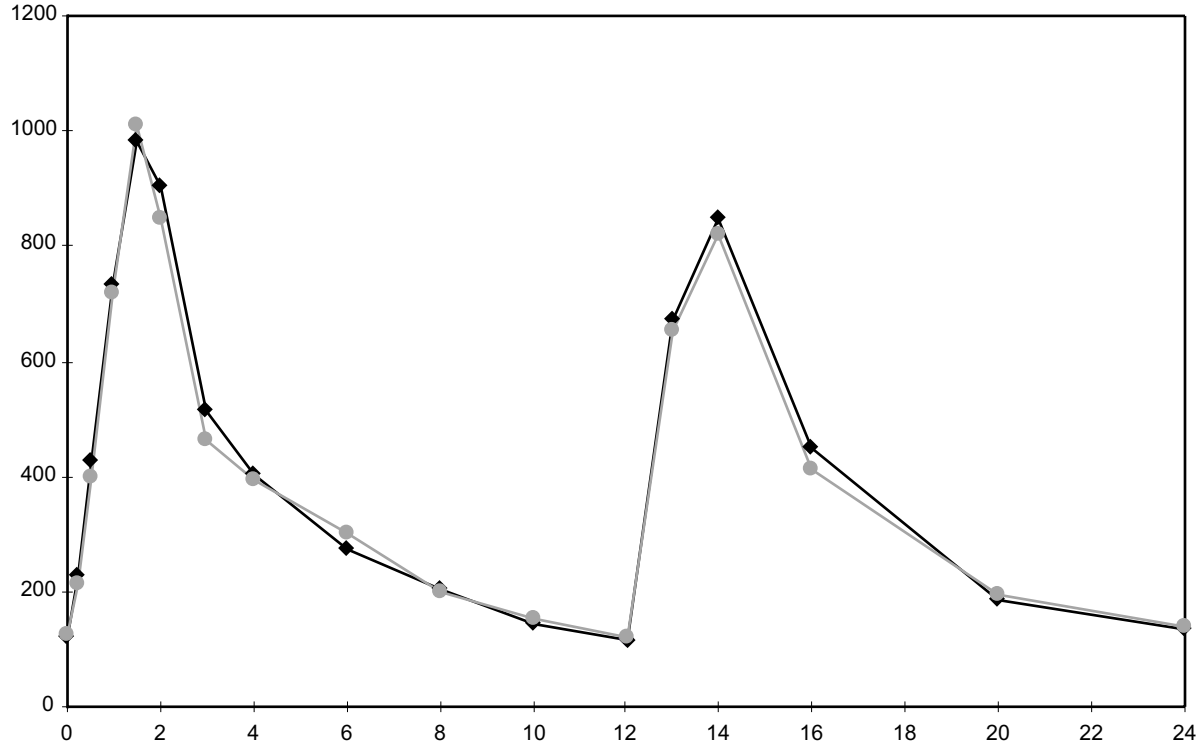

Figure 1. Concentration of cyclosporine in the whole blood after treatment with Test preparation ¢ and Reference preparation ¡ (mean values in ng/mL).

Table VII. Mean values (± SD) of pharmacokinetic parameters.

Treatments	AUC_{12-24}	$AUC_{0-\infty}$	C_{max}	t_{max}
Test preparation	4364.215 ± 688.953	5099.043 ± 825.086	924.834 ± 137.082	13.722 ± 0.461
Reference preparation	4233.914 ± 673.961	5038.420 ± 787.082	905.618 ± 173.767	13.778 ± 0.428

Table VIII. Statistical evaluation - P values of the ANOVA.

p-value	Log AUC_{12-24}	Log $AUC_{12-\infty}$	Log_{max}
Sequence	0.790	0.590	0.602
Treatments	0.114	0.395	0.448
Periods	0.961	0.998	0.936

Table IX. Statistical evaluation - t max of the Wilcoxon test.

	p-value
t max	0.317 ns

Table X. Statistical evaluation - mean values of pharmacokinetics parameters and C.I. 90% according to Westlake.

	Log AUC_{12-24}	Log $AUC_{12-\infty}$	Log C_{max}
Test preparation	3.635	3.702	2.962
Reference preparation	3.621	3.697	2.948
Lower limit	0.998	0.989	0.962
Test preparation/Reference preparation	103.157	101.135	103.181
Upper limit	1.065	1.034	1.107

Table XI. Statistical evaluation - P values of the ANOVA (differences [Δ], computed as logarithms, of cyclosporine blood levels of Test preparation and Reference preparation at various times).

	Δ 0 vs 12 hours	Δ 1 vs 13 hours	Δ 2 vs 14 hours
Sequence	0.703	0.292	0.259
Treatments	0.602	0.541	0.601
Periods	0.614	0.166	0.651

Table XII. Statistical evaluation - mean values of pharmacokinetic parameters and CI 90% according to Westlake.

	Δ Log 0 vs 12 hours	Δ Log 1 vs 13 hours	Δ Log 2 vs 14 hours
Test preparation	-0.0185	-0.0336	-0.0215
Reference preparation	-0.0238	-0.0425	-0.0112
Lower limit	0.972	0.964	0.903
Test preparation/Reference preparation	101.233	102.068	97.638
Upper limit	1.054	1.081	1.056

Table XIII. Statistical evaluation - P values of the ANOVA (differences [Δ], computed as logarithms, of cyclosporine blood levels of Test preparation and Reference preparation.

	Δ AUC_{0-t}	Δ $AUC_{0-\infty}$	Δ AUC_{max}
Sequence	0.736	0.447	0.638
Treatments	0.607	0.898	0.510
Periods	0.896	0.847	0.967

Table XIV. Statistical evaluation -mean values of pharmacokinetics parameters and CI 90% according to Westlake.

	Δ AUC_{0-}	Δ $AUC_{0-\infty}$	Δ AUC_{max}
Test preparation	0.0044	0.0168	-0.0783
Reference preparation	-0.0013	0.0156	-0.0935
Lower limit	0.970	0.965	0.946
Test preparation/Reference preparation	101.307	100.286	103.569
Upper limit	1.058	1.042	1.134

expressed as logarithms) of cyclosporine blood levels at the two phases, 0-12 hours and 12-24 hours. No significant difference has been detected.

Table XII reports the averages of treatments and the 90% confidential limits for the above analysed variables. The 90% confidencial interval for AUC-ratio and C_{max}-ratio lays within the bioequivalence range of 0.80-1.25 and 0.7-1.43 respectively. The values of variables calculated according to Westlake are within the confidential limits of 20-25%, thus indicating the bioequivalence of the two preparations.

Table 15 reports the p values calculated, by the ANOVA test, on the differences of cyclosporine blood levels at the following times: 0 vs. 12th hour, 1st vs. 13th hour, and 2nd vs. the 14th hour.

No significant difference has been detected.

Table 16 reports the p values, computed by the ANOVA test, with log values of AUC_{0-24}, $AUC_{0-\infty}$, and C_{max}. The statistical evaluation has reported a significant difference for the comparison between phases ($p < 0.01$).

Discussion

The Test formulation and the Reference preparation show an overlapping pharmacokinetic profiles. No difference has been noted by comparing both the over the time of whole blood concentration curves, and the main calculated pharmacokinetic parameters: AUC_s, C_{max}, and T_{max}.

The values of variables calculated according to Westlake are within the confidential limits of 20-25%, thus indicating the bioequivalence of the two preparations both in the 1st Phase (0-12 hours) and in the 2nd Phase (12-24 hours).

The statistical difference pointed out as far

Table XV. Statistical evaluation - P values of the ANOVA according to cross-over model at repeated measurements.

	0 vs 12 hours	1 vs 13 hours	2 vs 14 hours
Sequence	0.619	0.435	0.211
Treatments	0.274	0.341	0.142
Periods	0.794	0.330	0.646
Times	0.119	0.070	0.179
Times* treatments	0.602	0.541	0.601

Table XVI. Statistical evaluation - P values of the ANOVA of the pharmacokinetic parameters (expressed as logarithms) at the intervals: 0-24 hours and 0-∞ hours.

p-value	Log AUC_{12-24}	Log $AUC_{12-\infty}$	Log_{max}
Sequence	0.876	0.820	0.285
Treatments	0.087	0.340	0.529
Periods	0.851	0.834	0.839
Phase	0.890	0.257	>0.01**
Phase* treatments	0.607	0.898	0.510

as the comparison "between phases" is not surprising because it is most likely related to the different times of evaluation and to the different drug clearance and metabolism during the night and the day, as already found by other investigators[10].

The use of microemulsions has strongly reduced the well known inter-subject variability of blood levels of cyclosporine frequently seen with conventional pharmaceutical preparations. As consequence, the posology with microemulsion preparations can be established with an acceptable accuracy.

No adverse effects have been observed by the clinicians or reported by the volunteers participating to the study.

The results of the present study allow to conclude that the two preparations are bioequivalent. This offers the possibility to choose between them with no meaningful difference in the expected clinical effect.

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