

Comparison of two microemulsion of cyclosporine A in healthy volunteers

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Abstract. – This study evaluated the bioequivalence of a new Cyclosporine A microemulsion formulation in comparison to the reference market standard.

Twenty-four adult healthy volunteers were randomised to receive the two Cyclosporin A microemulsion formulations, at a dose of 2.5 mg/kg, according to a cross-over design. Blood samples were taken before drug administration and at 12 points within 24 hours. Cyclosporine A whole blood concentrations were determined by HPLC.

The pharmacokinetic parameters AUC_{0-t} and $AUC_{0-\infty}$ were calculated by the trapezoidal rule, C_{max} and T_{max} were obtained directly from blood data. $AUCs$ and C_{max} were tested for bioequivalence after log transformation of data, differences for T_{max} were evaluated by the rank test of Wilcoxon for paired data. The 90% confidence interval ratio between tested/reference drug was 0.98 for AUC_{0-t} , 0.96 for $AUC_{0-\infty}$ and 1.01 for C_{max} . All of them were within the range of bioequivalence.

T_{max} was 1.60 ± 0.44 hours after test drug and 1.67 ± 0.48 after reference drug ($p = 0.27$, Wilcoxon test).

According to these results the two Cyclosporine A microemulsion formulations can be considered bioequivalent.

Key Words :

Cyclosporine A, Bioequivalence, Microemulsion, Pharmacokinetics, Immunosuppression.

Introduction

Cyclosporine A is a powerful immunosuppressant drug, used to prevent organ transplant rejection and to treat severe autoimmune diseases¹⁻². Although this drug is available for oral administration, its bioavailability shows wide intra- and inter-individual variations, ranging from 1% to 95%³. Due to its lipophilic nature, absorption of cyclosporine

A from the upper small intestine is a zero-order process and is strongly influenced by bile secretion, pancreatic exocrine secretion, food intake and gastrointestinal motility⁴. For this reason, many formulations have been proposed to improve the gastrointestinal absorption of cyclosporine A.

In 1996, a cyclosporine A formulation in a microemulsion concentrate, containing a surfactant, lipophilic and hydrophilic solvents and an organic solvent had been introduced in therapy⁵. The microemulsion is characterised by a droplet size < 100 nm and is absorbed, independently from other exogenous or endogenous factors, in a dose-related manner⁶⁻⁸. Very recently, a new cyclosporine A microemulsion, characterised by a high uniformity of droplet size (32 ± 1 nm) has been developed by Sigma Pharma (Brazil)⁹.

The aim of our study was to investigate in healthy volunteers the pharmacokinetics of this new cyclosporine A microemulsion formulation comparing its bioavailability with the standard market microemulsion formulation.

Subjects and Methods

Twenty-four healthy volunteers, 12 males and 12 females, took part to this study. The experiment was planned and performed according to the Helsinki declaration and its Tokio amendment. Before starting the trial, all subjects had given their written consent. The volunteers were between 21 and 40 years weighing from 52 to 90 kg, within 20% of their ideal body weight. Their medical histories were carefully evaluated and they had complete physical examinations before and after the study, including assessment of blood pressure and heart rate. All of them had rou-

tine laboratory analyses, including a pregnancy test in females. The subjects were taking no other medication concurrently and abstained from taking any other drug for two weeks prior the study.

Preparations

The test cyclosporine A microemulsion formulation*.

The reference preparation was purchased as a market**.

Experimental design

The study was performed according to a single dose, crossover randomised design. All subjects received cyclosporine A microemulsion at a dose of 2.5 mg/kg. All drug preparations were given at 8.00 AM, after 12 hours fasting, with 150 ml of natural water. There was a wash-out period of at least 14 days between treatments.

Blood sample collections

Venous blood samples were taken before drug administration and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours later. Samples were collected in EDTA-containing tubes, gently inverted several times, and frozen at -20°C. Each whole blood sample was immediately put in test tubes, labelled with a protocol number, initials of the subjects and time. Samples were stored at -20°C until tested. Determination of cyclosporine A concentrations was performed in a double-blind fashion after assignment of the code number according to a randomisation list.

Analytical procedures

A sensitive reversed-phase high performance liquid chromatographic (HPLC) assay was used to determine the amount of cyclosporine A in the whole blood. According to Hassam¹⁰, the drug was extracted with ethyl ether from the acid whole blood sample. The organic layer was separated and evaporated to dryness. The residue was dissolved in a mobile phase and analysed by HPLC.

Cyclosporine D was used as Internal Standard (IS). The method was linear between 25 ng/ml and 1500 ng/ml.

Pharmacokinetic parameters

The following pharmacokinetic parameters were measured:

AUC_{0-t} : area under the blood concentration-time curve from time 0 to time t, calculated by the trapezoidal rule, where t is the last concentration estimated.

$AUC_{0-\infty}$: area under the blood concentration-time curve calculated according to the formula:

$AUC_{0-t} + C_t/\beta$, where:

C_t : last concentration estimated

β : slope of the phase of elimination.

C_{max} : peak drug concentration, obtained directly from the data.

T_{max} : time to peak drug concentration, obtained directly from the data.

Statistical analysis

Results were expressed as mean values \pm SD. Analysis of variance (ANOVA) was performed on pharmacokinetic parameters AUCs and C_{max} , after logarithmic transformation, using the *general linear model* procedure. The 90% confidence intervals for the ratio between the test and the reference averages of AUC and C_{max} were calculated according to Westlake¹¹. For bioequivalence they should lie within the ratio 0.8-1.25 for AUCs and 0.7-1.43 for C_{max} . The level of significance of the test was $\alpha = 0.05$.

The following sources of variation were considered: sequence, periods and treatments.

The T_{max} was evaluated by a non parametric procedure, the Wilcoxon matched-pair rank test. A p value < 0.05 was considered significant.

Results

The mean blood cyclosporine A levels following administration of test and reference preparation are shown in Table I. The resulting pharmacokinetic parameters (means \pm SD) are reported in Table II.

*SIGMASPORIN MICROORAL solution, by Sigma Pharma, Brazil.

**SANDIMMUN NEORAL solution, by Sandoz, Switzerland.

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Table I. Mean values \pm SD of cyclosporine concentrations (ng/ml) after administration of two preparations.

Time (hours)	Test preparation	Referent preparation
0	0	0
0.5	331.34 \pm 178.76	269.16 \pm 130.43
1	616.10 \pm 289.67	743.76 \pm 289.04
1.5	951.46 \pm 246.26	944.33 \pm 243.64
2	896.75 \pm 240.87	789.74 \pm 270.51
3	668.72 \pm 245.97	648.20 \pm 240.26
4	487.85 \pm 208.26	538.16 \pm 206.76
5	407.79 \pm 182.56	442.14 \pm 150.09
6	347.07 \pm 169.22	300.73 \pm 118.20
8	240.84 \pm 126.57	195.09 \pm 90.02
10	102.99 \pm 41.89	112.60 \pm 51.00
12	57.79 \pm 26.19	79.62 \pm 27.17
24	31.27 \pm 22.00	38.58 \pm 25.81

Significant amounts of the drug were found thirty minutes after administration. The two preparations were found to be not significantly different with respect to peak blood concentrations (1048.34 \pm 216.67 ng/ml for test drug vs 1036.95 \pm 214.20 ng/ml for reference drug) and to peak concentration time (1.6 \pm 0.44 h vs 1.67 \pm 0.48 h). After test preparation administration, the mean AUC_{0-t} was 4939.24 \pm 1652.44 ng/ml x h and $AUC_{0-\infty}$ was 5172.22 \pm 1736.68 ng/ml x h. There were not significant differences in comparison to the reference preparation (AUC_{0-t} = 4981.19 \pm 1584.04 ng/ml x h, $AUC_{0-\infty}$ = 5308.85 \pm 1682.96 ng/ml x h. Analysis of variance performed on these data after logarithmic transformation did not show any significant difference for sequence, periods and treatments. The 90% confidence intervals for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} ratios were 0.98, 0.96 and 1.01 (test drug/reference drug).

There were no significant differences in T_{max} at the Wilcoxon test for paired data ($p = 0.27$).

All volunteers accomplished their participation in the study in good health. No side ef-

fects were observed. Physical examinations and clinical laboratory tests performed after the study were unchanged and within the range of normality.

Discussion

Pharmacokinetic of cyclosporine A in humans is largely unpredictable; after oral administration, 2-fold intraindividual and 3-fold interindividual variations in the AUC are usually found¹². Absorption rate, first-pass metabolism and biliary excretion influence cyclosporine A bioavailability. Drug absorption is a zero order process and is strongly influenced by the fat content in the small intestine, bile secretion, gastrointestinal motility and drug administration. Furthermore patients subjected to liver transplantation with biliary diversion via a T-tube show even higher variations⁴.

To improve the gastrointestinal absorption of cyclosporine A, many pharmaceutical formulations have been proposed. A microemul-

Table II. Mean values \pm SD of pharmacokinetic parameters regarding two preparations.

	$AUC_{(0-t)}$ ng/ml/h	$AUC_{(0-\infty)}$ ng/ml/h	AUC_{max} ng/ml/h	T_{max} h
Test preparation	4939.24 \pm 1652.44	5172.22 \pm 1736.68	1048.34 \pm 216.67	1.60 \pm 0.44
Referent preparation	4981.19 \pm 1584.04	5308.85 \pm 1682.96	1036.95 \pm 214.20	1.67 \pm 0.48

sion seems to be highly effective as long as it has self-emulsifying properties and capacity to form a homogeneous microemulsion with droplet size < 100 nm. In microemulsion with such characteristics, cyclosporine A is dispersed in a mixture of a hydrophilic and lipophilic solvents. A surfactant and an antioxidant complete the formulation⁵.

In healthy volunteers, the cyclosporine A microemulsion, in a dose ranging between 300 and 800 mg, allows a reduction of T_{max} with respect to emulsion, between 13% and 62%, an increase of C_{max} between 16% and 104%, and an increase of AUC between 14% and 135%¹³⁻¹⁵. Furthermore, the interference of food assumption is strongly reduced. Improvement of pharmacokinetics have been demonstrated also in kidney, liver and heart transplant recipients⁵.

Progress in laser light scattering techniques^{16,17} has made possible to develop a new cyclosporine A microemulsion formulation, characterised by a reduced size of droplets (= 32 ± 1 nm) and a higher uniformity of the particles in order to guarantee a complete dispersion of the microemulsion, with better availability of the peptide molecules for the small bowel window. The test drug microemulsion subsides these characteristics displaying good bioavailability in experimental animals⁹.

In our study we found that this new cyclosporine A microemulsion formulation was readily absorbed by the small intestine of human volunteers, with good pharmacokinetic parameters and reduced interindividual variations.

Although the dose used was low (2.5 mg/kg), the whole blood cyclosporine A concentrations were between 400 and 800 ng/ml for more than 5 hours, with a potential full therapeutic effect against organ transplant rejection.

We had also evidence that the peak plasma concentration, the AUCs and the peak concentration time recorded after the administration of the new cyclosporine A microemulsion did not differ from those obtained with the standard cyclosporine A microemulsion on the market. The 90% confidence intervals for the test/reference drug ratios were 0.98 for $\log AUC_{0-1}$, 0.96 for $\log AUC_{0-\infty}$ and 1.01 for C_{max} . All of them were in the interval 0.8-1.25, stated by FDA¹⁸ and CMCP¹⁹ recom-

mendations for establishing drug bioequivalence. Finally, under the experimental conditions used, there were no differences in terms of sequence and periods between treatments.

The results showed that the two cyclosporine A microemulsion formulations can be considered bioequivalent in healthy volunteers. Further studies are needed to establish whether or not pathological conditions, known for their interference on cyclosporine A absorption rate, may affect the bioavailability of this new microemulsion formulation.

References

- 1) KAHAN BD. Cyclosporine. *N Engl J Med* 1989; 321: 1725-1738.
- 2) NOBLE S, MORKHAM A. Cyclosporine A review of the Pharmacokinetic propitious, clinical efficacy and tolerability of a microemulsion based formulation (Neoral). *Drugs* 1995; 50: 924-941.
- 3) PTACHCINSKI RJ, VENKATARAMANAN R, BURCKART GJ. Clinical pharmacokinetics of cyclosporine. *Clin Pharmacokinet* 1986; 11: 107-132.
- 4) FAHR A. Cyclosporine clinical pharmacokinetics. *Clin Pharmacokinet* 1993; 24: 472-495.
- 5) FRIMAN S, BACKMAN. A new microemulsion formulation of cyclosporine. *Clin Pharmacokinet* 1996; 30: 181-193.
- 6) RITSCHEL WA, ADOLPH S, RITSCHEL GB, SCHROEDER T. Improvement of peroral absorption of cyclosporine A by microemulsions. *Meth Find Exp Clin Pharmacol* 1990; 12: 127-134.
- 7) LEE YJ, CHUNG SJ, SHIM CK. Bioequivalence of Neoplanta capsule to Sandimmune Neoral, microemulsion formulations of cyclosporine A in human subjects. *Intern J Clin Pharmacol Ther* 1998; 36: 210-215.
- 8) KOVARIK JM, MUELLER EA, VAN BREE JB, TETZLOFF W, KUTZ K. Reduced inter- intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. *J Pharm Sci* 1994; 83: 444-446.
- 9) DE BERNARDI M. Bioavailability of two oral formulations of cyclosporine microemulsion in dog and rabbit (In Press).
- 10) HASSAM MM. Al-Yahya in analytical profiles of drug substances. In: Floney K, ed. New York: Academic Press, 1987; vol 16: 145-206.
- 11) WESTLAKE WJ. Use of confidence intervals in analysis of comparative bioavailability trials. *J Pharm Sci* 1972; 61: 1340-1341.

- 12) LINDHOLM A, HENRICSSON S, LIND M, DAHLQVIST R. Intraindividual variability in the relative systemic availability of cyclosporine A after oral dosing. *Eur J Clin Pharm* 1988; 34: 461-464.
- 13) DREWE J, MEIER R, VONDERSCHER J et al. Enhancement of absorption of cyclosporine in man. *Br J Clin Pharm* 1994; 34: 60-64.
- 14) MUELLER EA, KOVARIK JM, VAN BREE JB et al. Improved dose-linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharm Res* 1994; 11: 301-304.
- 15) MUELLER EA, KOVARIK JM, VAN BREE JB et al. Influence of fat-rich meal on the pharmacokinetics of a new oral preparation cyclosporin in a crossover comparison with the market formulation. *Pharm Res* 1994; 11: 301-304.
- 16) DE GIORGIO V, PIAZZA R. Characterisation of microemulsion: laser light scattering method applied to cyclosporine-A preparations. *Acta Techn Leg Medic* 1999 (in press).
- 17) DE GIORGIO V, PIAZZA R. Light scattering in colloid and interface science. *Curr Op in Coll Interf Sc* 1996; 1: 11-16.
- 18) FDA 1992 GUIDANCE. Statistical procedures for bioequivalence studies using standard 2-treatment cross-over design. Rockville: Statement Under 21 CFR 10.90.
- 19) CPMP (COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS) WORKING PARTY ON THE EFFICACY OF MEDICAL PRODUCTS 1991. Brussels: Note for guidance: investigation of bioavailability and bioequivalence.