

Cyclosporine bioavailability of two physically different oral formulations

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Abstract. – To assess the comparative bioavailability of two cyclosporine capsule products with different pharmaceutical formulation an open randomized two-period cross over study was conducted in 24 healthy volunteers.

Our results, obtained from cyclosporine HPLC determination onto the whole blood samples collected, show that the test cyclosporine non-SMEDDS formulation was not bioequivalent cyclosporine SMEDDS formulation due to a statistically significantly lower absorption rate.

The outcome does not support free and full interchangeability in chronic stable graft recipients of the two products studied, unless validated clinical and laboratory conversion protocols for each kind of organ transplantation are enforce.

Key Words:

Cyclosporine, Microemulsions, SMEDDS, Bioavailability.

Introduction

Cyclosporine is a lipophilic polypeptide. For its immunosuppressive properties, it is orally effective to prevent graft rejection in patients submitted to organ transplantation. Nevertheless, cyclosporine is a “critical” drug due to the narrow haematic concentration range at which its therapeutic effect takes place. When blood concentrations reach levels above this range, toxic effects – particularly nephrotoxicity – are seen.

On the other hand, below this range, it becomes ineffective leading to acute or sub-acute rejection crises.

In the eighties, the original standard commercial oral preparation was an oil solution

that was working by generating a macroemulsion, when coming in contact with the biliary flow. Because of the low aqueous dispersability of its active principle, this formulation was not ideal, leading to a slow and unhomogeneous absorption of cyclosporine from the small intestine¹.

The enteric oral absorption of cyclosporine in oleous macroparticle dispersions is a process dependent on bile salt secretion. Furthermore, many physiological and pathological conditions, such as timing and quality of food intake, interruption of enteric-hepatic circulation (especially after liver transplantation)², slow gastric emptying, diarrhoea, chronic and acute enteritis, gut's acute graft-versus-host disease, intestinal autonomic neuropathy and alterations of gastroenteric functionality among others, can significantly reduce cyclosporine absorption.

These conditions can cause a considerable inter- and intra-patients variability in gastrointestinal absorption. For instance, Lindholm and Kahan found that cyclosporine bioavailability after oral administration of the standard macroparticle emulsion varies between 20 and 60% of the given dose and increases with time after kidney transplantation³.

During the nineties, an innovative formulation technique named Self Micro Emulsifying Drug Delivery System (SMEDDS) was successfully applied to oral cyclosporine administration. According to Farah et al.⁴, a SMEDDS is a drug carrier consisting of natural or modified oils, surfactant and co-surfactant mixtures, liquid at room temperature, which emulsifies spontaneously when mixed with water (at 37° C). This new formulation favourably modifies the absorption kinetic of cyclosporine.

The most interesting feature of these admixtures is their ability to instantaneously generate a microparticle or micellar emulsion (simply called microemulsion) in an aqueous medium, such as the gastric fluids, so that drug dispersion and bioavailability are significantly improved. An increasing number of reports in the literature suggest that lipid-based microemulsions can be used to enhance the oral bioavailability of hydrophobic drugs including peptides⁵⁻⁷.

The SMEDDS technique, applied to cyclosporine, originated a new formulation that readily forms a homogeneous monophasic microemulsion, mimicking the physiological micellar phase of dietary lipids' absorption. The formation of this phase from SMEDDS formulations, does not appear to rely on emulsification by biliary salts and can happen regardless to bile secretion, which was not the case of the standard macroparticle emulsion used in the eighties⁸.

As a matter of fact cyclosporine absorption rate, after oral administration in the pharmaceutical form of monophasic microparticle emulsions is greater and more uniform in healthy volunteers or transplanted patients, under fasting conditions or with different diets and with or without alterations of gastrointestinal functionality⁹⁻¹³.

As a consequence, intra- and inter-subjects variability of drug oral bioavailability was becoming significantly lower with SMEDDS, leading to a reduction of the sample size necessary for testing bioequivalence between two different products¹⁴⁻¹⁸.

Recently, a new cyclosporine oral formulation contained in hard gelatine capsules has been put on the market. However, according to patent's data filed and scientific literature, this is a non-SMEDDS formulation – physically, a semisolid opaque oily suspension – that, in aqueous environment, generates a biphasic solid particle dispersion with a mean diameter well above 200 nm, approximately 7-fold higher than the average 30 nm-particle diameter induced by cyclosporine SMEDDS formulations¹⁹.

Study Objective

The purpose of this study was to compare the bioavailability of the reference oral SMEDDS cyclosporine formulation in soft

gelatine capsules with the recently marketed oral non-SMEDDS cyclosporine formulation in hard gelatine capsule on a population of 24 human healthy volunteers that is adequate for testing bioequivalence of cyclosporine microemulsions.

Materials and Methods

Subjects

Twenty four healthy volunteers were enrolled. The study was carried out in agreement with EEC and GCP rules. Before starting the trial, all volunteers signed informed consent. Before and after enrolment, routine clinical laboratory analyses including medical examination with SAP/DAP pressure and HR were checked.

Experimental Design

The study was conducted as an open randomized two-period cross over design, under fasting conditions. Each volunteer received a 200 mg single oral dose of each treatment with 150 ml of natural water, observing a 14-day washout period between treatments.

Blood Sample Collection

Five ml of venous blood were withdrawn into vacutainer tubes at the following times: 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours after treatment.

Samples were collected in EDTA-containing tubes. Whole blood samples were immediately put in test tubes, labelled with protocol number, subject's initials and time of collection. Samples were stored at -20° C until laboratory analysis.

Analytical Procedures

A sensitive reverse-phase high performance liquid chromatographic (HPLC) method was used to determine the amount of cyclosporine in human whole blood samples. The experimental procedure consisted of extraction of the drug with ethyl ether from the acid whole blood sample. The organic layer was separated, evaporated to dryness and the residue dissolved in mobile phase and analyzed by HPLC. Cyclosporine D was used as Internal Standard. The linearity of the method was evaluated in the range of concentration between 25 and 1500 ng/ml.

Pharmacokinetic Parameters and Statistical Analysis

Individual AUC_{0-t} , $AUC_{0-\infty}$, C_{max} , T_{max} , $t_{1/2}$, β and K_e were calculated. Results are expressed as mean values \pm SD. Statistical analyses were carried out on the above-mentioned parameters.

Analysis of variance (ANOVA) was performed on AUC_s and C_{max} using the "general linear model" procedure, after logarithmic transformation.

Factors accounting for the following sources of variation were considered: sequence, subjects (in sequence), period and treatment.

The 95% confidence intervals for the ratio between test and reference mean values of AUC and C_{max} were calculated according to the WESTLAKE test for bioequivalence²⁰.

Results

Cyclosporine blood concentrations at each sampling time, after administration of each formulation, are reported in Table I being the relative graph curves shown in Figure 1. Pharmacokinetic parameters are comparatively reported in Tables II to V.

Table I. Cyclosporine blood levels ($\mu\text{g/ml}$): mean values \pm SD.

Times	SMEDDS cyclosporine	Non-SMEDDS cyclosporine
0	0	0
0.5	0.284 \pm 0.142	0.357 \pm 0.174
1	0.777 \pm 0.289	0.661 \pm 0.281
1.5	0.969 \pm 0.238	0.796 \pm 0.220
2	0.792 \pm 0.258	0.803 \pm 0.236
2.5	0.695 \pm 0.215	0.706 \pm 0.262
3	0.594 \pm 0.187	0.595 \pm 0.256
4	0.474 \pm 0.161	0.403 \pm 0.199
6	0.312 \pm 0.119	0.292 \pm 0.162
8	0.233 \pm 0.085	0.194 \pm 0.146
10	0.127 \pm 0.067	0.130 \pm 0.133
12	0.081 \pm 0.028	0.056 \pm 0.036
24	0.038 \pm 0.025	0.0121 \pm 0.018

Discussion

The results obtained confirm that cyclosporine is a quite critical drug. Its absorption, in fact, is influenced by many factors and different processes: particles dimension of the emulsion, the characteristics of the administered solu-suspensions, the dispersion of the drug's molecules in the small intestine, the individual variability of the presence of natural emulsifiers like the biliary salts; food consumption, etc.

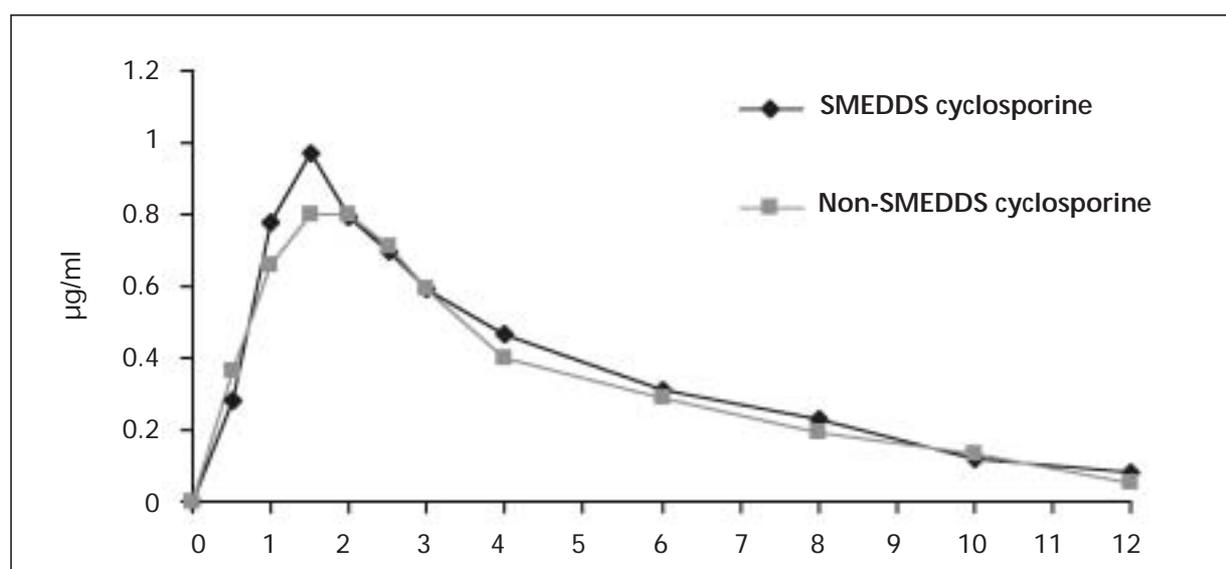


Figure 1. Cyclosporine comparative curves of mean blood levels ($\mu\text{g/ml}$).

Table II. Pharmacokinetic parameters: mean values \pm SD.

Parameters	SMEDDS	non-SMEDDS
AUC _{0-t} ($\mu\text{g. hrs. ml}^{-1}$)	4.971 \pm 1.436	4.196 \pm 1.517
AUC _{0-inf} ($\mu\text{g. hrs. ml}^{-1}$)	5.330 \pm 1.514	4.443 \pm 1.638
C _{max} ($\mu\text{g/ml}$)	1.025 \pm 0.213	0.873 \pm 0.207
T _{max} (hrs)	1.521 \pm 0.275	1.708 \pm 0.327
T _{1/2} β (hrs)	4.755 \pm 1.050	3.483 \pm 1.302
K _e	0.153 \pm 0.035	0.225 \pm 0.076

The use of a SMEDDS cyclosporine formulation increases the performance of the drug because of a better, faster and more regular absorption; in consequence of the self emulsifying activity, there is an ameliorative carriage of the drug into the blood, miming the one created into the micellar cells when the bile flow is naturally excited.

The non-SMEDDS cyclosporine formulation was not found bioequivalent to the SMEDDS reference formulation showing an overall, statistically significant, lower absorption rate.

It has to be emphasized that reaching the ideal oral dose of cyclosporine is a truly difficult task, as toxic effects (i.e., hyper dosage) and sub-therapeutic effects (i.e., hypo dosage) can lead to mixed adverse signs and symptoms.

Consequently, while “*de novo*” transplanted patients – who can take the test product since graft surgery undergoing frequent drug blood monitoring thereafter – may well be adapted to the test non-SMEDDS formulation, any subsequent change of the reference oral cyclosporine formulation being used in chronic stable transplanted patients has to be very carefully decided.

Although supply or economical reasons can strongly push for uncontrolled substitu-

Table III. Statistical evaluation (ANOVA): p-values.

	Log AUC _{0-t}	Log AUC _{0-inf}	Log C _{max}
Sequence	0.930	0.799	0.675
Treatments	0.071	0.050*	0.016*
Periods	0.385	0.375	0.437

*Statistically significant.

Table IV. Statistical evaluation: mean value of pharmacokinetic parameters and 95% CI according to Westlake.

	Log AUC _{0-t}	Log AUC _{0-inf}	Log C _{max}
SMEDDS mean values	0.678	0.709	0.0012
Non-SMEDDS mean values	0.594	0.618	-0.071
Lower Limit	0.667	0.658	0.742
Upper Limit	1.018	1.000	0.967
SMEDDS/ non-SMEDDS	82.41	81.13	84.66
Two one-sided test (p-value)	0.39*	0.45*	0.20*

*Not equivalent.

tion also in stable chronic transplanted patients, this can safely be done only through-out an accurate conversion protocol comprising at least weekly determinations of drug haematic levels, to find the optimal therapeutic dose with the new formulation.

Under this kind of scenario, strict equivalence between the physical forms of the two formulations to be interchanged during chronic treatments is a binding safety requirement to avoid unpredictable drifting drug absorption behaviours occurring in specific individuals or clinical subgroups, whenever frequent blood cyclosporine checking could not be enforced.

Our study shows that non-SMEDDS hard-gel capsules are not fully and freely interchangeable with SMEDDS soft-gel capsules, in the clinical setting.

Even if the dissolution profile could be similar, the pharmaceutical equivalence of the two products (test and reference) cannot be set because of their different physical form.

This study demonstrates that “*in vivo*” the non-SMEDDS cyclosporine capsules are not free and totally interchangeable with the SMEDDS formulated capsules of the reference drug.

Table V. Statistical evaluation of T_{max}: p-value according to Wilcoxon Test.

Non-SMEDDS T _{max} : 1.71 HRS	$p = 0.059$ n.s.
SMEDDS T _{max} : 1.52 HRS	

According to the variability coefficients calculated for AUC_s and C_{max} , that respectively are not included into the 0.80-1.25 and 0.70-1.43 intervals, the tested drug could not satisfy the approval criteria normally required for Generics.

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