

PHARMACOKINETICS OF CYCLOSPORINE FROM CONVENTIONAL AND NEW MICROEMULSION FORMULATIONS IN HEALTHY VOLUNTEERS

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ABSTRACT

The bioavailability of two microemulsion formulations of cyclosporine (Drug A, cyclosporine Neoral, Sandoz Pharma Ltd., Switzerland and Drug B, cyclosporine Bioral, Panacea Biotec Ltd., India) was compared in 12 healthy volunteers in a randomized, double blind, cross over manner. Blood cyclosporine levels were analyzed by high performance liquid chromatography. The peak concentration was 1600.63 ± 70.01 ng/ml with drug A and 1571.70 ± 65.40 ng/ml with drug B. The area under the concentration time curve (AUC $0 - \infty$) was 15321.69 ± 982.7 ng/ml and 13727.11 ± 722.90 ng/ml with drug A and drug B, respectively. These differences were statistically insignificant ($p > 0.05$). The other pharmacokinetic parameters of both the preparations were comparable suggesting that both the preparations are bioequivalent.

Also, the pharmacokinetics of cyclosporine from the new microemulsion was compared with the conventional formulation in normal healthy volunteers done in an earlier study. The C_{max} was greater with microemulsion (1571.70 ± 65.40) than conventional formulation (950.61 ± 83.99) and the variation in C_{max} between groups and within groups with microemulsion was 14.1% and 14.79%, respectively as compared to 31.75% and 31.95% with conventional formulation. The time to achieve C_{max} (t_{max}) was significantly shorter for microemulsion as compared to conventional formulation showing better bioavailability from microemulsion. (JAPI 1998; 46 : 860-3)

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INTRODUCTION

Cyclosporine is a cyclic undecapeptide with powerful immunosuppressive properties and is used as principal immunosuppressant agent in organ transplantation. The preferred route of cyclosporine administration is oral but the major disadvantage of orally administered cyclosporine is that its absorption is incomplete and can be both slow and erratic.¹ The pharmacokinetic properties of cyclosporine shows wide inter patient variation. The bioavailability of oral cyclosporine averages about 30%.¹ One of the contributory factors to this can be the release of drug from the currently marketed formulation.² The conventional oral formulation of cyclosporine is available as an oil based solution and the soft gelatin capsule. After oral administration, a crude oil-in-water droplet mixture is formed on contact with gastro intestinal fluids. Emulsification of this mixture by bile salts is required before digestion of the oily droplets and subsequently release of cyclosporine can occur. It is this emulsification step which makes the absorption of cyclosporine dependent on food intake, bile flow and gastro intestinal motility and therefore unpredictable. Once emulsification has been achieved the subsequent digestion process includes a mixed micellar phase in which small amounts of cyclosporine are solubilised in monoglycerides and bile salts.³

In an attempt to overcome the problems associated with cyclosporine mal absorption, a new oral microemulsion formulation (Panimun Bioral) has been developed for the first time in India. The new cyclosporine formulation is a microemulsion concentrate which consists of the drug in a lipophilic solvent and a hydrophilic solvent together with a surfactant and an antioxidant. On contact with gastrointestinal fluids this formulation readily forms a homogeneous, monophasic microemulsion which mimics the mixed micellar phase of cyclosporine absorption seen with conventional formulation. Because the formation of this phase does not appear to rely on emulsification by bile salts it has been suggested that absorption of cyclosporine from microemulsion formulation may be independent of bile flow. Since the drug has been manufactured for the first time in India, we conducted pharmacokinetic study and compared its bioavailability with already marketed microemulsion preparation Sandimun Neoral (Sandoz). Also we compared the pharmacokinetics of cyclosporine from new microemulsion with that from conventional formulation in normal healthy volunteers done in an earlier study.⁴

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MATERIAL AND METHODS

Experimental section

Subjects : Study was conducted in twelve healthy male volunteers aged 18-22 years. Written informed consent was obtained from all the volunteers. Volunteers were evaluated for general good health on the basis of medical history, routine biochemical and hematological tests, ECG and physical examination. They were asked not to take medication of any kind from least 1 week prior to the study and during

study period. Subjects also refrained from any strenuous exercise during the course of study

Study design

The study protocol was approved by ethical committee of the institute in accordance with the regulations of Drug Controller of India. The study was conducted in double blind, randomized cross over fashion and the successive administrations were separated by a wash out period of 2 weeks.

On the day of study, after an overnight fast zero hour blood sample was taken. Volunteers were then administered either of microemulsion of cyclosporine (Panimum Bioral or Sandimun Neoral) at the dose of 10 mg/kg with a glass of mango juice and the venous blood samples were collected for determination of cyclosporine in whole blood at 0.5, 1.0, 1.5, 2.5, 3.5, 4.5, 6.5, 9.5 and 24.0 hours in post dosing period. Samples were collected in EDTA containing tubes, gently mixed several times and frozen at -20°C till analysis. Standard breakfast and lunch were served after 3 and 6 hours, respectively and additional fluid and food intake was not allowed. Volunteers were monitored for development of adverse drug reactions (ADR) during the study period.

Drug assay

Concentrations of cyclosporine A in whole blood were assayed by high performance liquid chromatography method given by BIORAD, March 1993, using cyclosporine C as internal standard. Cyclosporine by this method is linear to 1000 ng/ml and levels as low as 40 ng/ml can be detected, giving peaks which are easily distinguished from background. The absolute recovery of cyclosporine A and internal standard was determined by addition of known amounts of each compound to cyclosporine free blood. Mean recovery for cyclosporine was 83%.

Pharmacokinetic parameters

Maximum whole blood concentration (Cmax) and time to achieve Cmax (tmax) were calculated from concentration-time curve data. The area under the concentration time curve (AUC 0-24) was calculated to the last blood concentration and extrapolated to infinity (AUC 0-∞) by adding AUC 24-∞ to AUC 0-24 where AUC 24-∞ was calculated by

$$AUC_{24-\infty} = \frac{\text{concentration at 24th}}{K_{el} \text{ (elimination rate constant)}}$$

Other parameters calculated were elimination half life (t 1/2 el), constant of elimination (Kel), volume of distribution (Vd) and clearance (CL). Relative bioequivalence was determined by AUC 0-∞ (test) x 100/AUC 0-∞ (reference).⁵ Statistical analysis was done by ANOVA (one way analysis of variance) data. Values were considered significant when p ≤ 0.05. All calculations were performed with RSTRIP (Version 5.0) software package.⁶ Pharmacokinetic parameters of the microemulsion formulations were compared with the pharmacokinetic parameters of conventional formulation. The results of latter formulation have already been published.⁴

Between and within subject variation² - For each formulation, the ANOVA mean sum of squares associated with between subject (MSb) and within subject (MSw) variabilities of the primary pharmacokinetic characteristics were derived as follows : MSb by pooling the mean squares for subjects and subjects per group and MSw by pooling the variation due to periods and treatment with the residuals. The MS for each pharmacokinetic characteristic were subsequently compared between the two formulations by an F-ratio test to determine whether the variabilities were significantly different. As an additional measure of variability, the percent coefficient of variation (CV) was derived from the respective ANOVA mean squares as follows :

$$CV_b = \frac{\sqrt{MS_b - MS_w}}{R} \times \frac{100}{\text{Grand mean}} \quad (1)$$

$$CV_w = \frac{\sqrt{MS_w}}{\text{Grand mean}} \times 100 \quad (2)$$

Grand mean

In equations (1) and (2), CV_b and CV_w are the coefficients of variation association with between and within subject variability, R is the number of administrations and grand mean is the mean of all observations.

Statistical hypotheses were tested at 0.05 significance level.

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RESULTS

Pharmacokinetic parameters for the two microemulsion formulations of cyclosporine are shown in Table-1. Mean blood concentration Vs time curve for test and reference product is illustrated in Fig. 1. After administration of cyclosporine microemulsions at the dose of 10 mg/kg, the mean concentration-time profile of both test and reference product showed comparative pharmacokinetic data. There was no significant difference in any of the pharmacokinetic parameters. The relative bioavailability of test formulation (Panimun Bioral) was 89.6%. Four volunteers complained of mild gastric irritation and two volunteers complained of burning sensation in hand and feet with both the formulations of microemulsion.

Parameter	Microemulsion (10 mg/kg)	
	Reference	Test
C _{max} (ng/ml)	1600.63 ± 70.01	1571.70 ± 165.40
t _{max} (h)	2.46 ± 0.31	2.29 ± 0.28
AUC 0-24 (ngh/ml)	12740.00 ± 499.60	12339.73 ± 665.40
AUC 0-∞ (ngh/ml)	15321.69 ± 982.70	13727.11 ± 722.90
t _{1/2el} (h)	10.50 ± 2.50	7.29 ± 0.63
K _{el} (h ⁻¹)	0.089 ± 0.01	0.10 ± 0.01
V _d (LAg)	9.36 ± 1.50	7.87 ± 0.67
CL (L/h)	0.68 ± 0.03	0.76 ± 0.05

Results are expressed as mean ± SEM

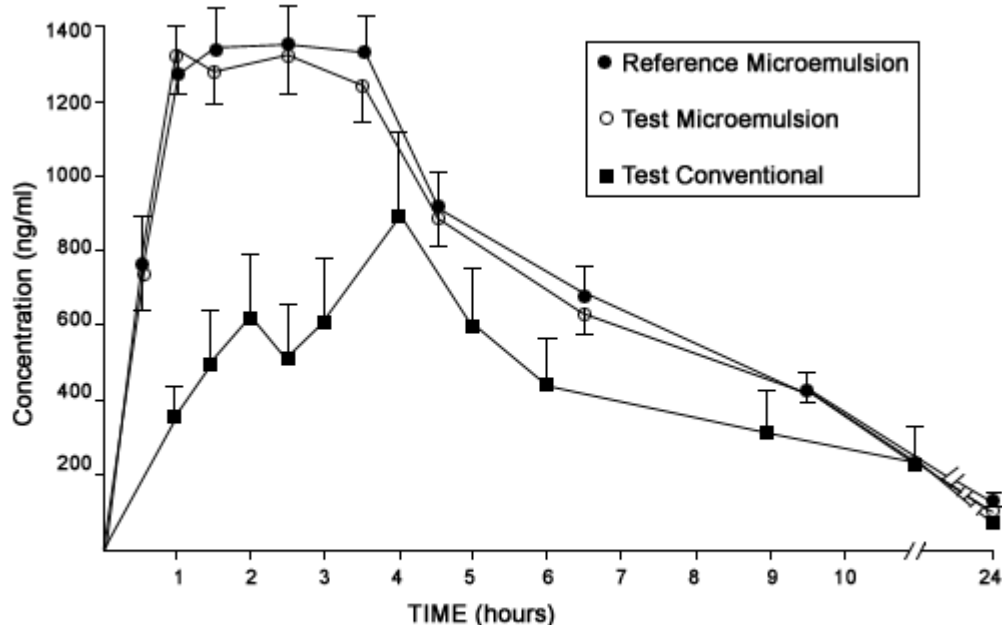


Fig. 1: Blood concentration - time curve of reference microemulsion, test microemulsion and test conventional formulations of cyclosporin in healthy volunteers.

Table 2 shows the comparative pharmacokinetic profile of microemulsions and conventional formulations (Panacea) of cyclosporine. Both maximum concentration (C_{max}) and area under the concentration-time curve measured in 24 hours following administration of microemulsions were consistently greater when compared with conventional formulation. There was no significant difference in the variation between the groups and within the group in both conventional and microemulsion formulation- The variation in C_{max} between groups and within groups with microemulsion was 14.1% and 14.79%, respectively as compared to 31.75% and 31.95% with conventional formulation. Also, the time to achieve C_{max} (t_{max}) was significantly shorter for the microemulsion when compared with the conventional formulation.

Parameter	Microemulsion (10mg/kg)	Conventional (15 mg/kg)
C _{max} (ng/ml)	1571.70±65.40	950.67 ± 83.99
t _{max} (h)	2.29 ± 0.28	4.25±0.65
AUC ₀₋₂₄ (ngh/inl)	12339.73 ±665.40	7301.87±546.69
AUC _{0-∞} (ngh/ml)	13727.11±722.90	10743.98±1287.94
t _{1/2 el} (h)	7.29 ± 0.63	11.89± 1.70
K _{el} (h ⁻¹)	0.10±0.01	0.128 ±0.064
VD (L/kg)	7.87 ± 0.67	24.41 ±3.18
CL (L/h)	0.76±0.05	1.63 ±0.21

Results are expressed as mean ± SEM

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DISCUSSION

The major concern with the use of cyclosporine is its less bioavailability and the factors which may affect bioavailability include malabsorption, food that may delay and impair absorption and enterohepatic recirculation.⁷ Various pharmacological strategies have been proposed to overcome the poor and erratic absorption of cyclosporine from conventional oral formulation.

Cyclosporine A is oil soluble and once dissolved in oil for pharmaceutical stability does not partition out in vivo therapy. As a result, there is incomplete and erratic absorption. The new self emulsifying

delivery system disperses cyclosporine A as microemulsion and hence increases surface area of absorption leading to better bioavailability.

We have shown in our study that rate and extent of cyclosporine absorption from microemulsions formulation is significantly greater than that from conventional formulation in 12 healthy male volunteers. The t_{max} occurred 1.96th earlier and C_{max} was over 1.65 folds higher at lesser dose of 10 mg/kg against 15 mg/kg dose of conventional formulation. AUC 0-24 with microemulsions was also nearly 1.7 folds greater when compared with conventional for initiation.

In order to minimise the influence of food intake on cyclosporine absorption from different oral formulations standard breakfast and lunch were served 3 and 6 hours after the drug administration. Another reason for less bioavailability of cyclosporine is its first pass metabolism and cyclosporine is the substrate for CYP3A4 enzyme. However, recent studies of cyclosporine disposition indicate that in addition to its metabolism in the liver. CYP3A4 metabolic activity in intestinal mucosa may substantially contribute to overall first pass effect.⁸ Contrary to the results reported by earlier study we did not find significant difference in inter as well as intra individual variation with both the formulations of cyclosporine. From the present study it is evident that microemulsion formulation has greater C_{max} , AUC, and shorter t_{max} even with lower dose of cyclosporine thus lower concentration of cyclosporine can be used to achieve the therapeutic response which would reduce its toxicity.

The incidence of side effects was greater with microemulsion than conventional formulation as none of the volunteers had reported any side effect with conventional formulation and with microemulsions formulation two of the volunteers complained of burning sensation in hand and feet. This could probably be attributed to increased C_{max} and shorter t_{max} , achieved with microemulsion.

In conclusion, this study has demonstrated that the microemulsion manufactured in India is as bioequivalent to the already marketed preparation and also there is improved absorption characteristics of new oral microemulsions formulation of cyclosporine in healthy male volunteers and a more consistent and predictable concentration time profile in comparison to conventional formulation.

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