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Research paper

Comparison of two cyclosporine formulations in healthy Middle Eastern volunteers: bioequivalence of the new Sigmasporin Microoral and Sandimmun Neoral

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Abstract

A study was conducted to establish bioequivalence between two oral cyclosporine 100 mg capsules, Sigmasporin Microoral (Gulf Pharmaceutical Industries – Julphar, United Arab Emirates, under technical co-operation with Sigma Pharma, Brazil) and Sandimmun Neoral (Novartis, Switzerland), in a Middle Eastern population, even though both formulations were found to be bioequivalent earlier in a Brazilian population (data on file). It was designed as a randomized, open label, two-way crossover study in which 30 fasting, healthy male volunteers received a single 100 mg cyclosporine dose with 240 ml of water on two treatment days separated by a 1 week washout period. After dosing, serial blood samples were collected for a period of 24 h. Plasma was analyzed for cyclosporine A by a sensitive, reproducible and accurate HPLC method with MS detection capable of detecting cyclosporine A in the range of 5–400 ng/ml with a limit of quantitation of 5 ng/ml. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ_Z were determined from plasma concentrations of both formulations. AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence intervals (82.98–110.57% for AUC_{0-t}, 81.57–124.71% for $AUC_{0-\infty}$, 80.15–98.91% for C_{max}) for these parameters were found to be within the bioequivalence acceptance range of 80–125%. Based on these statistical inferences, it was concluded that Sigmasporin Microoral® is bioequivalent to Sandimmun Neoral®.

Keywords: Cyclosporine; Bioequivalence; Pharmacokinetics; High performance liquid chromatography; Julphar

1. Introduction

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. The area under the concentration time curve (AUC) generally serves as the characteristic of the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}) reflect the rate of absorption, especially in fast releasing drug formulations [1]. In the present study bioequivalence of two cyclosporine A soft gelatine capsules filled with microemulsion was evaluated in Middle Eastern volunteers by comparing those pharmacokinetic parameters derived from the plasma concentration of cyclosporine A.

Following oral administration, the absorption and metabolism of cyclosporine A are highly variable from patient to patient, which is the result of poor gastrointestinal absorption [2]. Contributing factors to this poor absorption include a relatively large molecular weight, poor aqueous solubility and the presence of an absorption window in the small intestine [3]. A significantly enhanced oral bioavailability is observed when cyclosporine A is administered in a microemulsion (with bile acids) [2]. Following oral administration, the time to peak blood cyclosporine A concentrations (T_{max}) ranged from 1.5 to 2.0 h. Cyclosporine A is extensively metabolized by the cytochrome P-450 3A enzyme system in the liver, and to a lesser degree in the gastrointestinal tract, and the kidney; the metabolites have much less biological activity than the parent compound. Only 0.1% of a cyclosporine A dose is excreted unchanged in the urine. Elimination is primarily biliary with only 6% of the dose (parent drug and metabolites) excreted in the urine. The

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elimination half-life of cyclosporine A is highly variable, and reported to be in the range of 5–20 h [2].

2. Materials and methods

2.1. Study products

Test product	Sigmasporin Microoral® – cyclosporine 100 mg capsules
Batch No.	0606, Expiry: 11/2002
Manufacturer	Gulf Pharmaceutical Industries – Julphar, United Arab Emirates (under technical co-operation with Sigma Pharma, Brazil)
Reference product	Sandimmun Neoral® – cyclosporine 100 mg capsules
Batch No.	D03, Expiry: 10/2002
Manufacturer	Novartis, Switzerland

2.2. Study subjects

Thirty healthy adult male volunteers participated in this comparative study at Al-Mowasah Hospital, Amman, Jordan, as a joint venture with the International Pharmaceutical Research Centre (IPRC), Amman, Jordan. Their mean age was 25.4 ± 5.21 years with a range of 18–35 years; mean body weight was 70.7 ± 8.94 kg with a range of 55–87 kg and mean body height was 171.17 ± 6.12 cm with a range of 162–186 cm. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and haematological diseases, as determined by their medical history, physical examination, and routine laboratory tests (haematology, blood biochemistry, and urine analysis). All subjects were negative for hepatitis B antigen and were instructed to abstain from taking any drug including over-the-counter (OTC) drugs for 2 weeks prior to and during the study period. They were informed about the risks and aim of the study by the clinical investigator, based on which they signed a written informed consent statement before entering the study. The study was performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects and the rules of Good Clinical Practices. Before the start of the study the protocol was approved by the Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman, Jordan.

2.3. Drug administration and sample collection

The volunteers were hospitalized at 6:00 p.m. and had a standard dinner in hospital. After overnight fasting (10 h) subjects were given a single dose of either formulation (reference or test in a randomized fashion) of cyclosporine capsule with 240 ml of water. Food and drinks (other than water, which was allowed after 2 h) were not allowed until 5 h after ingestion of the capsule and then standard breakfast, lunch and dinner were given to all volunteers according to a time schedule. Beverages and food containing caffeine were not permitted over the entire course of the study. Volunteers sat or walked around during the first 5 h of blood collection, and were prohibited from strenuous activity. They were under direct medical supervision at the study site. Approximately 10 ml of blood samples for cyclosporine A assay were drawn into evacuated glass tubes (containing EDTA as anticoagulant) through indwelling cannula before (0 h) and at 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 h after dosing. Plasma was separated by centrifugation at 3500 rev./min for 5 min and stored frozen at −20 °C pending drug analysis. Vital signs, BP, pulse rate and temperature, were recorded at 0, 4, and 10 h. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

2.4. Sample preparation for HPLC injection

A total of 100 μl of the internal standard solution (cyclosporine B 5.0 μg/ml) was added to 1.0 ml of plasma and then the samples were vortexed for 30 s. A solid phase extraction technique was used (using HLB cartridges); 1.0 ml of methanol was added to the SPE column for conditioning, 1.00 ml of de-ionized water was added for equilibrium, the prepared plasma sample was added, 1.0 ml of de-ionized water was added for washing and then 1.50 ml of methanol was added for elution. The elute was evaporated to dryness at 50 °C under nitrogen gas, then reconstituted with 100 μl of mobile phase and transferred to a microglass (250 μl) tube and then centrifuged for 2 min at 13000 rev./min. Aliquot sample (50 μl) was injected into a Symmetry C8 (5 μm) (150 × 3.9 mm) HPLC column (Waters, Ireland) at 60 °C, where cyclosporine A and internal standard were separated from endogenous plasma substances.

2.5. Chromatographic conditions

Plasma samples were analyzed for cyclosporine A concentration according to a sensitive, selective and accurate HPLC method with LC-MS detection and validated before the study. All solvents used were of HPLC grade; other chemicals and reagents were of analytical grade; cyclosporine A was obtained from Julphar, United Arab Emirates and cyclosporine B was purchased from Galena.

The LC-MS system (ThermoQuest, UK) consisted of a mass detector Finnigan AQA, solvent delivery pump (P-2000), a system controller (SN 4000), an auto-sampler (AS 3000), and a switching valve (PR700-1000-01 – LabPRO, Rheodyne, USA). Integration was done using Xcalibur version 1.1 software (Finnigan, ThermoQuest, UK). The solid phase extraction apparatus was from Waters, USA, while the column oven (CTO-10A VP) was from Shimadzu, Japan. The mobile phase consisted of 75% acet-

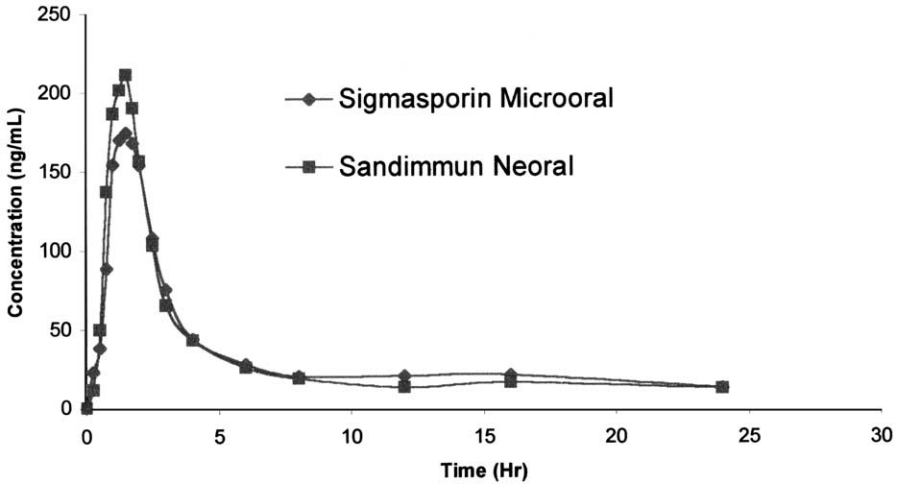

Fig. 1. Mean plasma concentrations of cyclosporine A after oral administration of two brands to 30 healthy human volunteers.

onitrile and 25% glacial acetic acid solution (10%) and elution of mobile phase followed a gradient program at 60 °C. The effluent was monitored using mass detection at ESI +ve (SIM) 601.3 and 594.4 for cyclosporine A and cyclosporine B, respectively. Each analysis required less than 12 min. The method was validated by following international guidelines [4].

2.6. Pharmacokinetic/statistical analysis

Pharmacokinetic analysis was performed by means of a model independent method using the Kinetica™ 2000 computer program [5]. The elimination rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. Elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_Z$, where C_t is the last measurable concentration. Statistical analysis was done using two-way analysis of variance (ANOVA GLM procedure; Kinetica™ 2000 Computer program) [5] for crossover design to assess the effect of formulations, periods, sequences and subjects on AUC and C_{max}. A difference between two related parameters was considered statistically significant for a P value equal to or less than 0.05. Parametric 90% confidence intervals [6] based on the ANOVA of the mean test/reference (T/R) ratios of AUC and C_{max} were computed.

3. Results and discussion

Both formulations were well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur.

The described analytical method was proven sensitive and accurate for determination of cyclosporine A in plasma. Under the described conditions, the lower limit of quantitation was 5.0 ng/ml using 1.0 ml of plasma. All validation parameters were within the acceptable limits. Both formulations were readily absorbed from the gastrointestinal tract and cyclosporine A was measurable at the first sampling time (0.25 h) in some volunteers while it was measurable at 0.5 h in almost all the volunteers. The mean concentration-time profile of the two formulations is shown in Fig. 1 indicating that the mean plasma drug concentration profiles of the two brands were closely similar and superimposable. The area under the curve up to infinity ($AUC_{0-\infty}$) could not be calculated for five and six volunteers in test and reference products, respectively, due to non-linear terminal phase.

The extent of absorption is a key characteristic of the drug formulation, and therefore AUC is an important parameter for comparative bioavailability (bioequivalence) studies. However, the other two parameters, C_{max} and T_{max}, are

Table 1
Pharmacokinetic parameters of cyclosporine 100 mg capsules (mean ± standard deviation, $n = 30$)

Pharmacokinetic parameter	Sigmasporin Microoral® (test)	Sandimmun Neoral® (reference)
AUC_{0-t} (ng/ml h)	561.07 ± 297.27	588.20 ± 247.07
$AUC_{0-\infty}$ (ng/ml h)	588.60 ± 328.04 ($n = 25$)	570.25 ± 235.12 ($n = 24$)
C_{max} (ng/ml)	226.98 ± 79.34	253.65 ± 85.31
T_{max} (h)	1.43 ± 0.38	1.44 ± 0.41
$T_{1/2}$ (h)	3.04 ± 2.34	2.41 ± 1.41
λ_Z (/h)	0.4083 ± 0.3484	0.4473 ± 0.3611

Table 2
Statistical analysis of log-transformed data

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM *P* value	0.9424	0.6058	0.0754
90% confidence interval	82.98–110.57%	81.57–124.71%	80.15–98.91%

also important features of the plasma level profile and could affect the therapeutic use of a drug and hence were also considered in the study.

The mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} (Table 1) of the two products did not differ significantly, suggesting that the blood profiles generated by Sigmasporin Microoral® are comparable to those produced by Sandimmun Neoral®. ANOVA for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having a *P* value greater than 0.05 (Table 2). Ninety percent confidence intervals (Table 2) also demonstrated that the ratios of AUC_{0-t} or $AUC_{0-\infty}$ or C_{max} of the two formulations lie within the FDA acceptable range of 80–125% [6]. The relative bioavailability of Sigmasporin Microoral® was 111.21% for AUC_{0-t}, 117.06% for $AUC_{0-\infty}$, and 93.08% for C_{max}.

For T_{max} the parametric point estimate of difference (test − reference) was −0.01 h, and was found to be within the acceptance limits (±20% of reference mean).

In the present study bioequivalence of a newly developed generic soft gelatine capsule containing cyclosporine A microemulsion was checked with that of the innovator's product. The existence of ethnic variations in the bioavailability of cyclosporine was proposed by Lindholm et al. in 1992 [7]. Based on that other authors demonstrated a difference in cyclosporine pharmacokinetics between Mexican and white volunteers [8] but this comparison was made using historical controls as the study did not include a control group of white individuals. Cyclosporine is biotransformed by CYP3A4, which is the same isoform of CYP that metabolizes nifedipine, other dihydropyridines, midzolam, and several other drugs [2]. Many reported studies have proposed the interethnic variability of disposition of these drugs due to higher CYP3A4 activity in whites compared with South Asians and Mexicans [9]. Ducharme et al. [10] have suggested that differences in gut metabolism (CYP3A4 activity) play an important role in interindividual variability regarding the bioavailability of oral cyclosporine. The variable activity of this enzyme may be determined by genetic or nutritional factors, resulting in an impaired first-pass extraction. Though the bioequivalence of this Sigmasporin Microoral was demonstrated earlier (data on file) in Brazilian volunteers, due to the reported ethnic variations in the bioavailability of cyclosporine it was rather important to confirm the bioequivalence between the two formulations in a Middle Eastern population where the test product Sigmasporin Microoral is planned to be marketed.

Plasma levels may be used as surrogate parameters for clinical activity. Therefore, the data of this study, by providing appropriate statistical results, suggest equal clinical efficacy of the two brands of cyclosporine A in the tested Middle Eastern population.

Based on the above we can conclude that Sigmasporin Microoral® manufactured by Gulf Pharmaceutical Industries, United Arab Emirates is bioequivalent to Sandimmun Neoral® manufactured by Novartis, Switzerland and that both products can be considered equally effective and interchangeable in medical practice.

References

[1] H.U. Schulz, V.W. Steinijans, Striving for standards in bioequivalence assessment: a review, Int. J. Clin. Pharmacol. Ther. Toxicol. 30 (Suppl. 1) (1992) S1–S6.
[2] Product Information: Neoral(R), cyclosporine. Novartis Pharmaceuticals, East Hanover, NJ (PI revised 11/1999), reviewed 02/2001.
[3] Y.J. Lee, S.J. Chung, C.K. Shim, Bioequivalence of Neoplanta capsule to Sandimmun Neoral, microemulsion formulations of cyclosporin A in human subjects, Int. J. Clin. Pharmacol. Ther. 36 (4) (1998) 210–215.
[4] V.P. Shah, K.K. Midha, S. Sighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloft, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pitman, S. Spector, Analytical method validation: bioavailability, bioequivalence and pharmacokinetic studies, Eur. J. Drug Metab. Pharmacokinet. 16 (1992) 249–255.
[5] Kinetica™ 2000, Version 3.0, Innaphase, User Manual, 1999.
[6] US Food and Drug Administration, FDA Guidelines, Bioequivalence Food and Drug Administration, Division of Bioequivalence, Office of Generic Drugs, Rockville, MD, 1992.
[7] A. Lindholm, M. Welsh, C. Alton, B.D. Kahan, Demographic factors influencing cyclosporine pharmacokinetics parameters in patients with uremia: racial differences in bioavailability, Clin. Pharmcol. Ther. 52 (1992) 359–371.
[8] J.A. Palma-Aguirre, J. Gonzalez-Laven, J.F. Francisco, C.H. Gilberto, Bioavailability of oral cyclosporine in healthy Mexican volunteers: evidence for interethnic variability, J. Clin. Pharmacol. 37 (1997) 630–634.
[9] G. Castaneda-Hernandez, J.A. Palma-Aguirre, M.A. Montoya-Cabrera, J.F. Flores-Murreita, Interethnic variability in Nifedipine disposition: reduced systemic plasma clearance in Mexican subjects, Br. J. Clin. Pharmacol. 41 (1996) 433–434.
[10] M.P. Ducharme, L.H. Warbasse, D.J. Edwards, Disposition of intravenous and oral cyclosporine after administration with grapefruit juice, Clin. Pharmacol. Ther. 57 (1995) 485–491.