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TRANSPLANTATION PROCEEDINGS

Equoral[®], New Cyclosporine Drug Delivery System, Versus Neoral[®]: A Bioequivalence Study in Healthy Volunteers

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Transplantation Proceedings, 35, 207-209 (2003)

Equoral[®], New Cyclosporine Drug Delivery System, Versus Neoral[®]: A Bioequivalence Study in Healthy Volunteers

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CYCLOSPORINE is used primarily to prevent graft rejection after solid organ transplantation, for prophylaxis of graft-versus-host disease, and for treatment of autoimmune diseases. The physicochemical properties of cyclosporine, particularly its low water solubility, does not allow formulation as a simple preparation, hence, in the case of cyclosporine dosage forms, it is technically more precise to assess drug-delivery systems. The first conventional cyclosporine-containing dosage forms, characterized by variable and incomplete absorption from the gastrointestinal tracts, were introduced in clinical practice two decades ago. During the long-term period of cyclosporine dosage form development a number of significant findings have been presented in the field of drug-delivery systems. From a technological view point, the currently available dosage forms are based on self-dispersible systems, which contain cyclosporine dissolved in a pharmaceutically acceptable vehicle.¹ The improvement in bioavailability between the original emulsion-based formula and the modified microemulsion-based formula has been presented as simply a consequence of particle size reduction followed by improved size distribution.

The aim of this study was to investigate the effect of particle size on cyclosporine absorption by demonstrating that in healthy human volunteers, improved bioavailability may be obtained by the new gel base EM drug-delivery system (GEDDS) treatment. For this purpose, a pilot bioequivalence study was performed on 12 healthy volunteers. Two different formulations of the DDS (formulation A-GEM 101 and formulation B-GEM 304) were compared with Neoral[®] (formulation C) soft gelatin capsules containing 100 mg of cyclosporine. Despite the large difference in particle size, the tested products were bioequivalent.

MATERIALS AND METHODS

This open-label, randomized, three-period crossover study was designed for 12 white healthy volunteers, ranging in age from 18 to 45 years. The tested medications and reference product were administered in a randomized sequence as single oral doses under the fasting conditions. Each dose contained 200 mg cyclosporine (administered as two 100-mg soft gelatin capsules). The duration of the washout period was at least 7 days. In each study period, 14 blood samples were obtained as follows: before administration; at 20, 40, and 60 minutes; and at 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours after administration. Adverse effects were monitored throughout the study.

Blood samples were taken from the antecubital vein into EDTA-containing plastic tubes (Sarstedt Monovettes) and kept deep frozen until processing. Cyclosporine blood concentrations were determined by means of a specific RIA. The AUC (0→∞) and C_{\max} were determined as the primary variables for evaluation of bioavailability. AUC (0→ t), t_{\max} , $t_{1/2}$, MRT (0→ t), MRT (0→∞), and F_{rel} were calculated as secondary variables.

From the concentration/time data of the parent compound, the pharmacokinetic parameters were determined for each individual dataset by using noncompartment analysis with TOPFIT 2.0. C_{\max} and t_{\max} values were identified directly from the observed concentration-time data. The elimination rate constant (k_{el}) was calculated by log-linear least-squares regression analysis of the terminal part of the plasma concentration-time curve. The area under the concentration-time curve (AUC 0→ t) was calculated up to the last measurable concentration timepoint (t) by the linear trapezoid rule. Extrapolation to infinity (AUC 0→∞) was performed by dividing the last observed concentration by the elimination rate constant.

STATISTICAL ANALYSIS

Arithmetic means, standard deviations, and medians were calculated for concentrations time data. For t_{\max} and $t_{1/2}$ arithmetic means and standard deviations were calculated. In addition, 95% confidence intervals were calculated for the means. For C_{\max} , AUC (0→ t), AUC (0→∞), and F_{rel} , geometric means and standard deviations were calculated, and the 95% confidence intervals for the means were determined.

The primary variables of AUC (0→∞) and C_{\max} , after logarithmic transformation, were subjected to ANOVA with the factor subject, formulation, and period. For the evaluation of bioequivalency, 90% confidence intervals were calculated. The accepted bioequivalency ranges were 0.80 to 1.25 for the AUC and 0.70 to 1.43 for C_{\max} data.

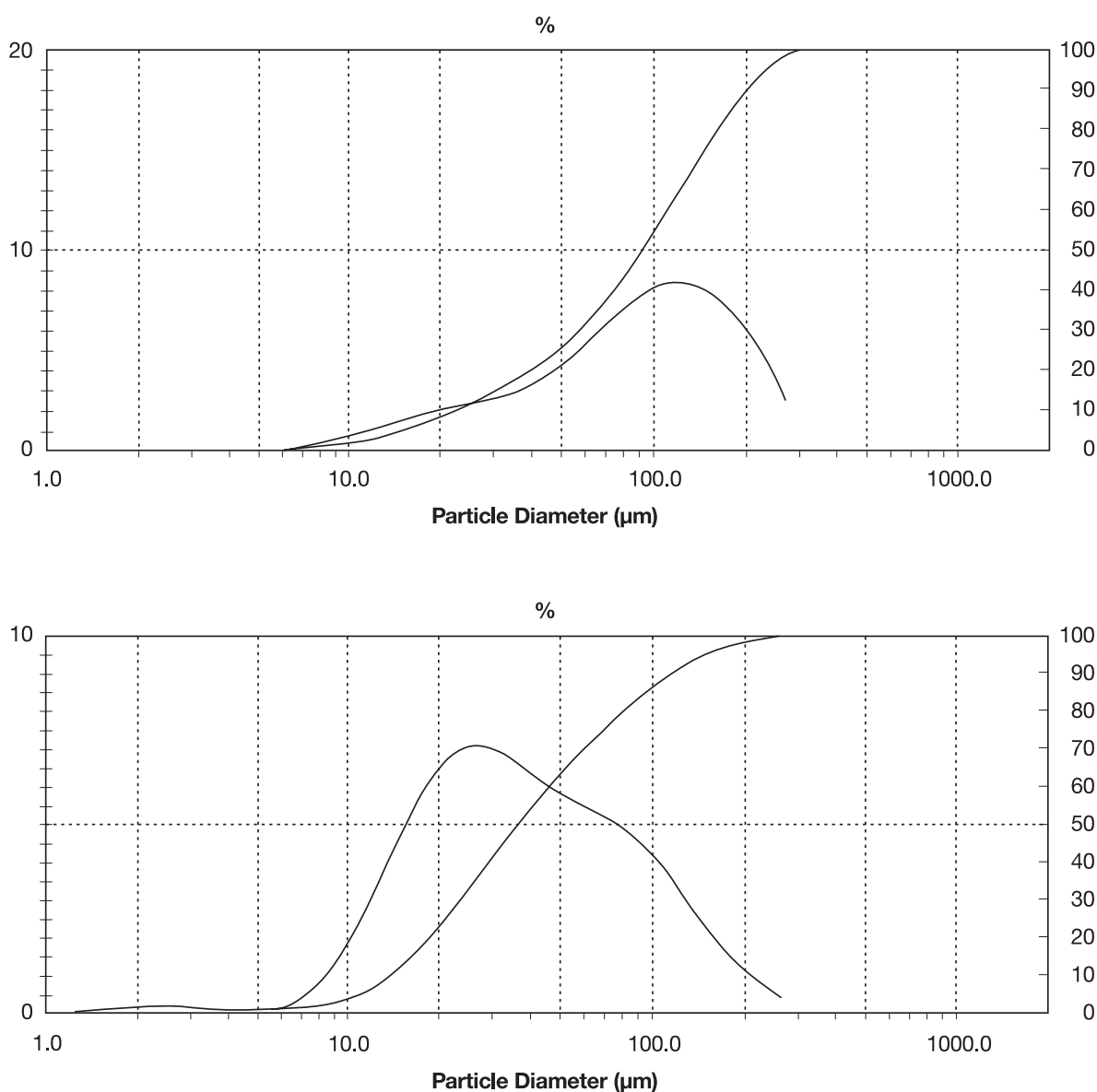
From IVAX-CR Opava, Czech Republic (T.A., A.J., V.M.); Rizk Hospital, Beirut, Lebanon (M.M.); and University Hospital, Hradec Kralove, Czech Republic (Z.V.).

Address reprint requests to Dr T. Andrysek, IVAX-CR AS, Ostravska, 747 70 Opava, Czech Republic.

0041-1345/03/\$—see front matter
doi:10.1016/S0041-1345(02)03924-6

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Fig 1. Histogram of particle size distribution. (a) Formulation A, GEM 101. (b) Formulation B, GEM 304.



PARTICLE SIZE EVALUATION AND VISUALIZATION

MASTERSIZER MICRO, v2.18 (Malvern Instruments, Ltd) was used for particle size distribution of the novel GEMDDS formulations, using purified water stirred to 14,000 rpm as a medium. All measurements were done at ambient temperature. The photomicroscope technique, a laboratory microscope–equipped color CCD camera with a picture analysis system (LUCIA, Laboratory Imaging), was used to visualize the particles. For GEMDDS dispersion, 0.15 g of the tested formula was poured into 10 mL of purified water and shaken vigorously for 10 seconds before visualization.

RESULTS AND DISCUSSION

Visualization

The visualization experiments showed that GEMDDS particles formed in the aqueous milieu kept their gel character. Contrary to standard emulsion spherical-shape droplets, they created stable nonspherical structures. As nonspherical particles are defined as those whose bear at least two diameters perpendicular to each other that are not equivalent. In accordance with this observation, GEMDDS was defined on the basis of dispersion of gel particles in the aqueous phase.

Particle Size

Fig 1. shows histograms of particle distribution of formulation A (GEM 101) and formulation B (GEM 304). The effective diameters deduced from the histogram were 92.05 µm for formulation A and 36.23 µm for formulation B.

Table 1. Summary of Pharmacokinetic Data

Parameter	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (ng/mL)	AUC (0→ t) (ng · h/mL)	AUC (0→ ∞) (ng · h/mL)
Formulation A					
Aritmetic mean	6.24	1.33	1372.69	4631.75	4861.85
SD	1.3	0.33	351.28	1204.56	1241.87
Geometric mean	6.12	1.3	1329.84	4483.35	4712.35
Minimum	4.06	1	908.1	2635.32	2873.57
Maximum	8.24	2	1930.3	6432.76	6684.33
Formulation B					
Aritmetic mean	6.41	1.5	1196.49	4430.33	4696.56
SD	1.3	0.48	308.26	1032.91	1143.13
Geometric mean	6.29	1.43	1161.84	4326.15	4576.94
Minimum	4.21	1	851.8	3130.66	3254.08
Maximum	8.93	2.5	1785	6206.13	6643.15
Formulation C / reference					
Aritmetic mean	6.13	1.33	1358.95	4647.01	4887.55
SD	1.32	0.33	380.35	1358.41	1430.5
Geometric mean	5.99	1.3	1307.19	4472.8	4705.55
Minimum	3.92	1	820.7	2953.47	3028.58
Maximum	7.87	2	1805.3	7330.08	7686.89

Pharmacokinetic and Bioequivalence Data

Absorption profiles of cyclosporine for all preparations were almost identical, illustrating the bioequivalence. Pharmacokinetic data are summarized in Table 1. The ANOVA indicated a statistically significant medication effect for all three variables with a significant period effect for AUC (0→ ∞) and AUC (0→ t) ($P < .05$). The 90% confidence limits for AUC and C_{max} were well within the accepted bioequivalence range compared with Neoral®. The percent coefficient of variation (%CV) for GEM 101 and GEM 304 were almost identical. The results of pharmacokinetic studies showed no apparent correlation between particle size and absorption profile or %CV.

These results clearly demonstrate the considerable potential of the GEMDDS to increase the oral bioavailability of the lipophilic drug, cyclosporine. Comparison of the effective size of particles created after dispersions showed formulations A and B gave approximately 1000-fold larger particles than the reference microemulsion. Biopharmaceutical experiments have focused on the correlation between particle size distribution and bioavailability in dogs and showed the same improved bioavailability, with systems giving particle size distributions near the submicron range.²

Consequently, no correlation between particle size distribution (molecular weight of aggregation and bioavailability) was observed when evaluating the model-microdispersed system with 141-nm particle average size.³

Based on current findings it is possible to deduce that particle size is not the only crucial point determining

bioavailability of hydrophobic drugs (eg, cyclosporine) from solution-based systems. The reasons for good bioavailability of cyclosporine from GEMDDS are both physical and physiologic/metabolic. The physical reason for the improved bioavailability from GEMDDS was seen in its ability to overcome an unstirred water layer⁴ in the lumen of a small intestine and adhere to the intestinal wall, ensuring both sufficient time and concentration gradient for absorption of cyclosporine. The metabolic interaction could be simplified as follows. All cyclosporine formulations contain nonionic surfactants, which probably affect permeation or absorption of cyclosporine through the intestinal wall. The effect of excipients on drug bioavailability has been documented since the early 1990s. Surfactants have been described to cause reversible local microvillar damage, which increases permeability through the intestinal wall.⁵ Another potential effect of excipients may be the disruption of tight junctions, observed in Caco-2 cells.⁶ In addition, some excipients are known to modify the activity of the multidrug resistance proteins — efflux transporter, MDR 1, also called P-glycoprotein (P-gp) and/or metabolic enzymes like cytochrome P450, subclass 3A4 (CYP3A4).⁷ All these mechanisms processes, together with others not yet described, probably represent mechanisms involved in intestinal absorption of hydrophobic drugs. Whatever process is directly responsible for cyclosporine absorption, we have shown that there is no direct or exclusive correlation between particle size and cyclosporine absorption.

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