Decreased oral availability of cyclosporin A at second administration in humans

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Aims The objective of this study was to determine the extent of period effect on the pharmacokinetics of cyclosporin A (CsA) during consecutive dosing.

Methods Sandimmune Neoral® and Neoplanta® capsules were administered to twenty-four healthy Korean male subjects at a single CsA dose of 175 mg in a 2 × 2 crossover investigation with a 2-week wash-out phase. Concentrations of CsA in blood were measured by a r.i.a. method for a period of 48 h.

Results The two formulations were found bioequivalent, but analysis of variance (ANOVA) indicated that there is a significant ($P < 0.01$) period effect in AUC(0,last) (area under the blood concentration–time curve above the assay limit) and $C_{\max}$ (maximum blood concentration) between the administrations. A 6 and 9% decrease in the AUC(0,last) and $C_{\max}$, respectively was seen at the second administration.

Conclusions This period effect on the pharmacokinetics of CsA may be relevant for the patients who need consecutive administration of the drug.

Keywords: CsA, period effect, bioequivalence

Introduction

A bioequivalence test of Sandimmune Neoral® (Sandoz Pharma, Ltd) and Neoplanta® (Hanmi Pharm. Co.) capsules, microemulsion formulations of cyclosporin A (CsA), was performed in 24 healthy Korean male volunteers [1]. The two formulations were administered as single doses in a 2 × 2 crossover design with a wash-out phase of 2 weeks between the administrations. The two formulations were equivalent with respect to peak CsA concentration in blood ($C_{\max}$), time to reach the peak ($t_{\max}$) and area under the concentration time curve in blood above assay limit AUC(0,last) when evaluated according to the Korean Guidelines of Bioequivalence Test (KGBT) [2]. However, from analysis of variance (ANOVA) for these parameters, a significant ($P < 0.01$) period effect was observed for AUC(0,last) and $C_{\max}$ between the administrations [1]. This study describes this effect and its extent. This effect has not been reported for CsA in the literature.

Methods

Formulations

Sandimmune Neoral® (25 and 100 mg as CsA; lot no. 070MFD0795 and 017MFD0795, respectively, Sandoz Pharma, Ltd, Basle, Switzerland) and Neoplanta® (25 and 100 mg as CsA; lot no. 144001 and 145001, respectively, Hanmi Pharmaceutical, Ltd, Seoul, Korea) were supplied as soft gelatin capsules.

Subjects

Twenty-four healthy male Korean volunteers, ranging in age from 23 to 33 years (25.6 ± 2.2), in weight from 61 to 74 kg (67.2 ± 4.5 kg), and in height from 165 to 185 cm (174.3 ± 5.4 cm) completed the study. Volunteers were selected after passing a clinical screening procedure including a routine physical examination and laboratory tests. All subjects avoided using other drugs for at least 1 week prior to the study and until after its completion. They also refrained from alcoholic beverages and caffeine containing food and beverages 48 h prior to each dosing and until the collection of the last blood sample.

Study implementation

The study was performed in accordance with KGBT [2]. Each subject received a 175 mg dose of CsA in a randomized, two-way crossover investigation. There was a 2 weeks wash-out phase between the doses. Sandimmune Neoral® (one 100 mg and three 25 mg capsules) and Neoplanta® (one 100 mg and three 25 mg capsules) were administered orally.

Subjects fasted from 10 h before drug administration until 4 h after. The doses were taken at 09.00 h on each dosing day with 200 ml of tap water. At 4, 12 and 22 h after administration, all subjects were given standardized meals. Subjects were not allowed to remain in a supine position or to sleep during the experiment for up to 12 h. Venous blood samples (8 ml) for the determination of CsA were obtained pre-dose and then 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, and 48 h after administration. Samples were obtained from a forearm by venepuncture through an intravenous catheter (21 G Green Cross, Seoul) and collected

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in EDTA-containing glass tubes (Green Cross, Seoul), gently inverted several times, and frozen at -20°C until the analysis.

Bioanalytical assay of CsA in whole blood

Concentrations of CsA in whole blood were assayed with the commercially available CYCLO-Trac[®] SP-whole blood radioimmunoassay (r.i.a.) kit (Incstar Co., Stillwater, MS) that is based on the use of a monoclonal antibody specific for the parent compound [3]. All the blood samples were assayed in a random order to prevent any possible period effect of the assay. The overall detection limit calculated from the mean concentration corresponding to 95% binding was $8.5 \pm 1.2 \text{ ng ml}^{-1}$. The limit of the quantification (LOQ) was 12.5 ng ml^{-1} with $<20\%$ of coefficient of intra-assay variation.

Pharmacokinetic and statistical analyses

Noncompartmental pharmacokinetic characteristics were derived by standard methods. Extrapolated area in AUC (i.e., $\text{AUC}(\text{last}, \infty)$), calculated by the log-linear trapezoidal rule, was generally less than 10% of total area (i.e. $\text{AUC}(0, \infty)$) for all cases. Thus, the $\text{AUC}(0, \text{last})$ was used instead of AUC extrapolated to time infinity as recommended by Martinez & Jackson [4]. C_{max} and t_{max} were read directly from the concentration-time data. Analysis of variance (ANOVA) was performed for $\text{AUC}(0, \text{last})$, C_{max} and t_{max} , according to KGBT guidelines. Differences in the bioavailability parameters between the administration periods were examined for their significance with the paired t -test using SPSS[®] (SPSS Inc., Illinois, U.S.A.).

Results

Sandimmune Neoral and Neoplanta were found bioequivalent when evaluated according to the KGBT (i.e., the 90% confidence intervals of the differences (δ) in C_{max} , $\text{AUC}(0, \text{last})$ and t_{max} between the two formulations were $< \pm 20\%$).

The ANOVA table (Table 1) revealed that there were no significant F -values for group/sequence (between subjects) and formulations (within subjects) for all the parameters.

However, significant F -values were observed for subject/groups (between subjects for all parameters) and for periods (within subjects for $\text{AUC}(0, \text{last})$ and C_{max}). This observation indicates a substantial intersubject variation for all parameters and a significant ($P < 0.01$) period effect for $\text{AUC}(0, \text{last})$ and C_{max} between the administration periods. The period effect could be seen clearly when the bioavailability parameters from each administration were compared in each subject (Figure 1). In most of the subjects in both groups, the $\text{AUC}(0, \text{last})$ and C_{max} from the period II were lower than those from the period I. As a result, the mean $\text{AUC}(0, \text{last})$ decreased significantly from 4270 ± 1120 to $3990 \pm 1130 \text{ ng ml}^{-1} \text{ h}$ and C_{max} from 1290 ± 218 to $1170 \pm 217 \text{ ng ml}^{-1}$ of the 24 subjects ($P < 0.01$, 6 and 9% reduction), with the repeated administration. No difference was found for t_{max} between the administration periods (1.47 ± 0.33 and $1.52 \pm 0.31 \text{ h}$, respectively).

Figure 1 Inter-periodic comparison of $\text{AUC}(0, \text{last})$ (upper panel) and C_{max} (lower panel) of cyclosporin A in each subject for group A ($n=12$, left column) and B ($n=12$, right column). P values were generated from a paired t -test using SPSS[®] (SPSS Inc., Illinois, U.S.A.).

Source of variation	DF	AUC (0, last)		C _{max}		t _{max}	
		MS	F	MS	F	MS	F
Between subjects							
Group/sequence	1	108459	0.06	320	0.00	0.220	1.52
Subject/groups	22	2535579	22.99★	78557	4.30★	0.145	2.32★
Within subjects							
Periods	1	896683	8.13★	176096	9.64★	0.033	0.52
Formulations	1	161707	1.47	40080	2.20	0.001	0.02
Residuals	22	113704		18270		0.062	

C_{max} : peak blood concentration, t_{max} : time to reach C_{max} , $\text{AUC}(0, \text{last})$: area under the blood concentration over assay limit of the quantification-time curve, DF: degree of freedom, MS: mean squares, F : distribution. $F_{(0.05) 1, 22} = 4.30$, $F_{(0.05) 22, 22} = 2.05$. * $P < 0.05$.

Table 1 Summary of ANOVA for bioavailability parameters.

Discussion

This study was designed to test a bioequivalence of two CsA formulations. Therefore, a 2×2 design was adopted in this study, instead of a three phase study, which would be more appropriate to examine the period effect. The 2×2 design, however, showed a small but significant decrease in AUC(0,last) and $C_{\max}$ at the second administration (Figure 1). In the literature, this period effect has never been reported for CsA and the underlying mechanism is unknown. The decrease in AUC(0,last) and $C_{\max}$ may be mediated by an increase in hepatic and/or gastrointestinal (GI) first pass metabolism and/or by a decrease in GI absorption at the second administration. A significant first pass metabolism was reported for CsA by cytochrome P-450 3A system in the liver and the GI membranes [6, 7]. Thus, a change in the first pass metabolism is likely to have a significant impact on CsA bioavailability as evidenced by a recent report [8]. In addition, a reduction in GI absorption for CsA at the second administration appears possible since a chronic oral dosing of CsA to rats up-regulated the expression of p-glycoprotein in the intestine [9], a protein that is thought to be responsible for an efflux process for CsA from the enterocyte into the intestinal lumen [10].

Clinical implication of the period effect is currently unknown. Better designed studies (e.g., a three phase study, multiple dosing study) appear necessary to fully realize the clinical impact of this observation. This aspect of CsA pharmacokinetics is under investigation in our laboratory.

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