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ORIGINAL ARTICLE

Neoimmun versus Neoral: a bioequivalence study in healthy volunteers and influence of a fat-rich meal on the bioavailability of Neoimmun

F. Kees · M. Bucher · F. Schweda · H. Gschaidmeier · L. Faerber · R. Seifert

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Abstract In two crossover studies with 12 (6 males/6 females) healthy young volunteers each, we compared the bioavailability of Neoimmun capsules with the microemulsion Neoral and the influence of a fat-rich breakfast on the bioavailability of Neoimmun. Each volunteer received a single dose of 200 mg cyclosporine A in each period. Blood samples were taken up to 24 h and analysed for cyclosporine A by high-performance liquid chromatography (HPLC) and photometric detection. The pharmacokinetic parameters were determined by non-compartmental analysis. The treatments were tested for bioequivalence and significant differences. The bioavailability of Neoimmun was significantly lower compared to Neoral, albeit Neoimmun met the bioequivalence criterion (90% confidence interval of AUC 0.80–0.94) or missed the criterion only marginally (90% confidence interval of c_{max} 0.75–0.91). The bioavailability of Neoimmun as determined by area under the blood concentration-time curve (AUC) increased by nearly 20% after a fat-rich breakfast. However, mean peak concentrations after food were only higher in male subjects, whereas mean peak concentrations in female subjects were lower compared to fasting administration. In conclusion, our data show that Neoimmun exhibits a lower bioavailability than the microemulsion Neoral and that food has a significant but variable and sex-dependent impact on the bioavailability of Neoimmun capsules.

Keywords Cyclosporine A · Food effect · Bioavailability · Bioequivalence · Gender effect

F. Kees (✉) · L. Faerber · R. Seifert
Department of Pharmacology and Toxicology,
University of Regensburg,
Universitätsstrasse 31,
93053 Regensburg, Germany
e-mail: Frieder.kees@chemie.uni-regensburg.de

M. Bucher
Department of Anesthesiology, University of Regensburg,
Regensburg, Germany

F. Schweda
Department of Physiology, University of Regensburg,
Regensburg, Germany

H. Gschaidmeier · L. Faerber
Novartis Pharma,
Nuremberg, Germany

Introduction

Cyclosporine A (CyA) is a critical dose drug with low water solubility, and many variables have been found to influence the bioavailability of oral CyA (Fleisher et al. 1999; Klauser et al. 1997; Wu and Benet 2005). In contrast to the original Sandimmune capsules, the microemulsion formulation Neoral provides a higher and more consistent bioavailability independent of food intake including fat-rich meals (Mueller et al. 1994) and has set a new standard for oral CyA medications (Ponticelli 2005). Based on bioequivalence studies, generic formulations of CyA have been approved as bioequivalent to Neoral such as Gengraf (Abbott, North Chicago, IL, USA), Cicloral (Hexal AG, Holzkirchen, Germany) or Neoimmun (Kwizda Pharma, Vienna, Austria), which is identical to Equoral (Ivax, Miami, FL, USA). However, in two independent crossover studies in healthy young volunteers, we found that the bioavailability of Cicloral was significantly lower compared to Neoral and was enhanced by more than 25% when administered after a fat-rich breakfast. The test of bioequivalence for Cicloral failed (Kees et al. 2004, 2006). The aim of the present study was to compare

the bioavailability of Neoimmun with Neoral as reference and to investigate the influence of a fat-rich breakfast on the bioavailability of Neoimmun.

Materials and methods

Study design

The study protocol was approved by the ethics committee of the University Hospital Regensburg. Written informed consent was obtained from all volunteers. Volunteers were evaluated for general good health on the basis of medical history, physical examination, electrocardiogram and routine laboratory tests. Both studies were performed according to an open, blockwise randomised two-period crossover design in 12 volunteers each. There was a 1-week washout between each treatment period of a study. In study A, all volunteers (median, range; 6 m, age 24, 22–25 years, body weight 73, 66–75 kg, body height 180, 172–188 cm, BMI 21.4, 21.2–24.7; 6 f, age 23, 22–30 years, body weight 58, 50–81 kg, body height 168, 158–174 cm, BMI 20.2, 19.1–26.8) received two capsules Neoimmun (test) and two capsules Neoral (reference) with 200 ml of water on an empty stomach. In study B, the volunteers (median, range; 6 m, age 24, 23–26 years, body weight 71, 66–76 kg, body height 183, 175–188 cm, BMI 21.5, 19.9–24.2; 6 f, age 24, 22–29 years, body weight 58, 52–63 kg, body height 170, 158–172 cm, BMI 20.5, 19.4–21.3) received two capsules Neoimmun after a fat-rich breakfast (test) and fasting (reference) with 200 ml of water. Each capsule contained 100 mg CyA. No other medications were permitted during the course of each study except contraceptives. Venous blood samples for the determination of CyA in whole blood were obtained before drug administration (time 0) and after 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 h. Samples were collected in EDTA-containing tubes and stored frozen at −30°C. In study A, drugs were administered after an overnight fast. Two hours later, a low-fat breakfast was served, lunch 4.5 h, a snack 7 h, and dinner 10 h after drug administration. In each period of study B, half of the volunteers performed the study as in study A and half of the subjects received the medication immediately after a fat-rich breakfast (two eggs fried in butter, two strips of fat bacon, 120 g hash brown potatoes, 250 ml of whole milk and two slices of toast with butter) according to the recommendation of the FDA (Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalence Studies, Dec. 2002, http://www.fda.gov/cder/guidance/index.htm#Biopharmaceutics). Smoking, alcoholic and caffeine-containing beverages were not allowed from 12 h before and up to 24 h after drug administration.

Drugs and chemicals

Neoimmun 100 mg capsules (batch no. 4T507002; expiry date 07/2008, Kwizda) and Neoral 100 mg capsules (Sandimmun® Optoral 100 mg capsules batch no. S0157, expiry date 07/2008, Novartis Pharma, Nuremberg, Germany) were obtained from a public pharmacy. CyA and CyD (internal standard) were a kind gift of Novartis Pharmaceuticals, Basel, Switzerland. Acetonitrile and methanol (ultra gradient HPLC grade) were purchased from Baker, Groß-Gerau, Germany; the other chemicals (analytical grade) from E. Merck, Darmstadt, Germany. Water was purified by a Milli-Q water purification system (Millipore, Eschborn, Germany).

HPLC assay

CyA was determined by high performance liquid chromatography (HPLC) and photometric detection at 205 nm as published previously (Kees et al. 2006) using a mobile phase with a slightly higher content of acetonitrile (water-acetonitrile-methanol 350:600:60, *v*/*v*/*v*). CyA eluted after 6.1 and CyD after 7.1 min at a flow rate of 1.1 ml/min. The lower limit of quantification (LLQ) was 10 ng/ml for CyA using 1 ml of whole blood. At quality control concentrations of CyA 1,000 (50) ng/ml, the intra-assay precision in study A was 4.0 (3.4)%, the inter-assay precision was 0.70 (2.9)% and the bias was −0.77 (−0.90)%. In study B, the respective values were: intra-assay precision 3.0 or 6.8%, inter-assay precision 2.0 or 4.7% and bias −0.60 or +0.08%.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of CyA were determined by standard non-compartmental methods. Maximum blood concentration (c_{max}) and time to peak concentrations (T_{max}) were directly obtained from the individual blood concentration-time curves. The elimination constant λ_z was calculated by log-linear regression in the elimination phase, typically from 8 to 24 h. The terminal half-life was calculated according to $t_{1/2}=\ln 2\ \lambda_z^{-1}$. The area under the blood concentration–time curve to the last quantifiable concentration (AUC_t) was calculated by the linear trapezoidal rule. The measured last concentration (c_t) was used for extrapolation to infinity to determine $AUC_\infty = AUC_t + c_t\,\lambda_z^{-1}$. The mean residual AUC from the last measurement point to infinity, as a percentage of total AUC, was between 5.0% (Neoimmun after fat-rich meal) and 5.7% (Neoimmun, study A). The dosage regimens were tested for bioequivalence using the statistical program BioQ PC V1.2.2 (obtained from Dr. Steinijans, Altana Pharma, Konstanz, Germany). Ninety percent confidence intervals were calculated according to the two-period crossover design using the residuals from an

appropriate analysis of variance. The range of bioequivalence (AUC, c_{max}) was set to 80–125% using the logarithmically transformed data (note for Guidance on the investigation of Bioavailability and Bioequivalence, Committee for Proprietary Medicinal Products: Commission of the European Communities, London, 26 July 2001). The no-difference hypothesis in T_{max} was accepted if the 90% confidence interval of the differences in T_{max} included the value "zero" (Steinijans VW 1993). In analogy to a previous study (Kees et al. 2004), we included 12 subjects in each study. A posterori power analysis (α=0.05) using the mean values and the SD for the obtained differences gave a power of 0.94 (c_{max} Neoral vs Neoimmun), 0.89 (AUC Neoral vs Neoimmun) and 0.92 (AUC Neoimmun fed vs fasting), respectively. The *t* test for paired data was used to compare the difference between two treatments, and the Mann–Whitney *U* test was used to evaluate differences between male and female subjects. *P* values less than 0.05 were considered statistically significant. Data are presented as means±SD when not otherwise indicated.

Results

Tolerability

Generally, the drugs and treatments were well tolerated. Four volunteers in study A and seven volunteers in study B reported seven and nine episodes of burning sensations (face, hands, feet or esophagus), respectively, shortly after drug intake and up to 3 or 4 h. Furthermore, three episodes of nausea were observed in study B where the drug was taken after food. Two episodes of headache in study B and one episode of headache, nausea and vomiting 12 hours after drug intake in study A were classified as non-drug-related.

Whole blood concentrations and pharmacokinetic parameters of CyA

The mean blood concentration–time profiles of CyA are depicted in Fig. 1. The mean blood concentrations of CyA after Neoimmun were lower compared with Neoral (Fig. 1a). The blood concentrations of Neoimmun increased after a high fat breakfast and showed a slight shoulder between 4 and 6 h (Fig. 1b). The bioavalability of Neoimmun was 86% compared to Neoral (Table 1) and increased after a fat-rich breakfast (Table 2). The increase in c_{max} after food was less pronounced compared to the increase in AUC.

The 90% confidence interval of the ratio Neoimmun (test) vs Neoral (reference) with respect to c_{max} as well as AUC was completely below 100%. Regarding AUC, the 90% confidence intervals were at the lower limit of bioequivalence (Neoimmun vs Neroal) or at the upper limit of bioequivalence (Neoimmun fed vs fasting). The 90% intervals regarding peak concentrations exceeded the range of 80–125%. Therefore, bioequivalence could not be demonstrated in the present study (Table 1).

Separate analysis of male and female volunteers revealed that female volunteers showed the tendency to a lower bioavailability. Mean peak concentrations as well as AUC related to 70 kg body weight were lower. The difference in the bioavailability of Neoimmun compared to Neoral was smaller, and only AUC increased after fat-rich breakfast in both groups (ratio fed/fasting: AUC 1.24±0.16 in male vs 1.12±0.15 in female subjects and c_{max} 1.40±0.28 vs 0.95±0.31). In addition, the half-life of CyA was apparently shorter in female subjects (Tables 1 and 2). The differences were significant regarding peak concentrations, half-life and AUC of Neoimmun after a fat-rich meal. Mean lower peak concentrations after the fat-rich breakfast and a shoulder in the blood concentrations between 4 and 6 h indicated a biphasic absorption process in female subjects (Fig. 2b).

Discussion

Based on bioequivalence studies, generic formulations of CyA have been approved as bioequivalent to Neoral such as Gengraf, Cicloral or Equoral, which is identical to Neoimmun (Ivax). However, in previous studies in 12 young healthy volunteers, we could not demonstrate bioequivalence, neither between Cicloral and Neoral nor between Cicloral under

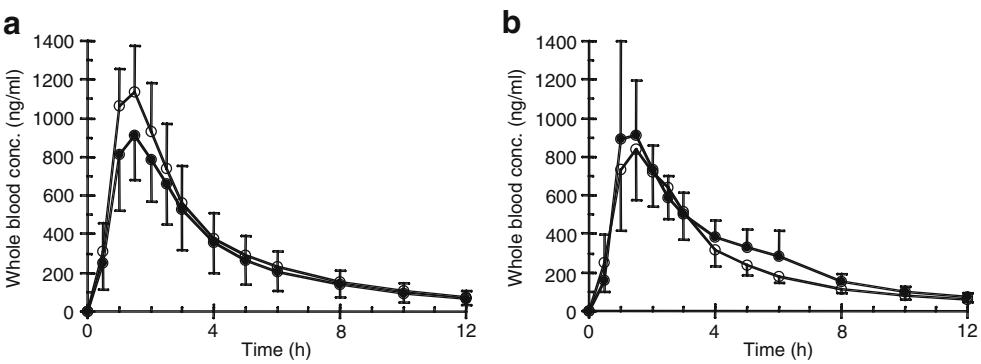

Fig. 1 Mean (SD) whole blood concentrations of CyA in 12 (6 males/6 females) healthy young volunteers after oral administration of two capsules Neoimmun (*closed symbols*) or Neoral (*open symbols*) fasting (**a**), and of **b** Neoimmun fasting (*open symbols*) or after a fat-rich breakfast (*closed symbols*). Each capsule contained 100 mg CyA

Table 1 Pharmacokinetic parameters of CyA in 12 healthy young volunteers (6 males/6 females) after oral administration of two capsules Neoimmun (test) or Neoral (reference) and test of bioequivalence (study A)

Subj.	Sex	BW (kg)	c_{max}/70 kg (ng/ml)		T_{max} (h)		$t_{1/2}$ (h)		AUC/70 kg (ng/ml·h)	
			Nim	Neo	Nim	Neo	Nim	Neo	Nim	Neo
1	F	60	739	923	1.5	1.5	7.1	7.2	3,725	4,175
3	F	50	845	914	2.0	1.5	6.7	6.5	5,060	5,136
7	F	56	867	1,243	1.5	1.5	3.6	2.7	3,100	3,644
8	F	60	1,066	1,207	1.0	1.5	6.2	6.2	4,251	4,552
10	F	54	802	762	1.0	1.0	6.3	6.2	2,732	2,991
11	F	81	919	1,119	1.0	1.0	3.4	3.9	2,743	3,666
2	M	73	1,350	1,457	1.5	1.5	7.7	7.2	7,673	7,767
4	M	73	872	979	1.0	1.0	8.0	7.7	3,188	3,174
5	M	75	959	942	2.0	1.5	7.3	6.7	4,989	5,011
6	M	66	875	1,247	1.5	1.0	2.9	3.4	2,739	4,096
9	M	73	1,110	1,432	1.5	1.0	6.0	5.9	4,369	5,338
12	M	72	728	1,304	1.5	1.0	6.1	6.7	3,351	4,935
Mean		66.1	928	1,127	1.42	1.25	5.93	5.85	3,993	4,541
SD		9.8	176	222	0.36	0.26	1.72	1.62	1,431	1,277
PE, 90% CI			0.83, 0.75–0.91						0.86, 0.80–0.94	
P			0.005						0.003	
Female subjects, n=6										
Mean		60.2	873	1,028	1.33	1.33	5.54	5.45	3,602	4,027
SD		10.9	112	190	0.41	0.26	1.61	1.76	928	759
Male subjects, n=6										
Mean		72.0	982	1,227	1.50	1.17	6.33	6.26	4,385	5,054
SD		3.1	219	221	0.32	0.26	1.89	1.52	1,811	1,543
P			0.298	0.066			0.576	0.337	0.472	0.230

Each capsule contained 100 mg CyA.
Nim Neoimmun, *Neo* Neoral, *SD* standard deviation, *PE* point estimate, *CI* confidence interval

fasting conditions and after a fat-rich breakfast (Kees et al. 2004, 2006). In particular, the bioavailability of Cicloral was significantly lower compared to Neoral and increased by more than 25% after a fat-rich breakfast.

In the present investigation with Neoimmun, we made similar observations as previously with Cicloral. Under fasting conditions, the mean bioavailability of Neoimmun was significantly lower compared to Neoral. The 90% confidence intervals of the ratios of AUC as well as of c_{max} were totally below 100% compared to Neoral, albeit Neoimmun met the bioequivalence criterion (AUC) or missed the criterion only marginally (c_{max}). Like Cicloral or the original Sandimmune oily suspension, the bioavailability of Neoimmun increased significantly when administered after a fat-rich breakfast. However, sub-analysis of male and female subjects revealed that in female subjects, only AUC increased (and less than in male subjects), whereas mean peak concentrations were even lower after the fat-rich breakfast. The difference between male and female subjects shows that the influence of food on the bioavailability of Neoimmun varies, thereby impeding therapeutic drug monitoring using in particular peak blood concentrations (C2) as surrogate parameter, which is believed to be superior to trough levels (C12; Johnston et al. 2003; Marin et al. 2006; Morris et al. 2002; Oellerich and Armstrong 2002, 2006).

Nonetheless, our studies revealed significant differences in the bioavailability between Neoimmun and Neoral and between Neoimmun fasting and after a fat-rich breakfast. The primary aim of our studies was not to demonstrate bioequivalence, but we emphasize that we would have probably found bioequivalence between Neoimmun and Neoral or Neoimmun fed vs fasting using more subjects, rendering the 90% confidence interval more narrow, as the point estimates of c_{max} as well as of AUC were between 80 and 125%. Bioequivalence between Equoral (Neoimmun) and Neoral has been reported in a previously published study in 12 healthy volunteers according to the authors' statement (Andrysek et al. 2003). However, the experimental setting of the study and the test medication were not precisely described, and the result of the bioequivalence test was not provided. Similar limitations are also applicable to a more recent review on the bioequivalence of Neoral and Equoral (Masri et al. 2005). However, unpublished studies (data on file, Equoral product monograph, Ivax) have demonstrated bioequivalence between Equoral and Neoral

Table 2 Pharmacokinetic parameters of CyA in 12 healthy young volunteers (6 males/6 females) after oral administration of two capsules Neoimmun after a fat-rich breakfast (test) or fasting (reference), and test of bioequivalence (study B)

Subj.	Sex	BW (kg)	c_{max}/70 kg (ng/ml)		T_{max} (h)		$T_{1/2}$ (h)		AUC/70 kg (ng/ml·h)	
			Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting
101	F	57	804	913	1.0	1.0	5.5	6.8	3,558	3,820
103	F	58	595	575	2.0	3.0	5.7	6.1	3,649	2,921
105	F	63	700	814	1.5	2.5	5.2	6.7	3,272	3,389
107	F	56	553	660	2.0	2.0	5.6	7.1	3,847	3,255
109	F	60	1,262	840	1.0	1.5	8.1	8.3	4,171	3,215
111	F	52	588	1,027	2.5	1.5	5.1	6.2	3,759	3,500
102	M	70	1,140	879	1.0	1.0	6.0	7.5	4,661	3,717
104	M	76	1,313	881	1.0	1.5	9.1	7.7	5,079	4,436
106	M	75	1,172	1075	1.0	1.0	6.4	6.7	3,460	3,299
108	M	74	1,099	978	1.5	1.0	6.8	6.0	4,403	3,852
110	M	66	1,331	842	1.5	1.5	7.0	6.6	4,531	3,342
112	M	68	1,563	856	1.0	1.5	8.1	7.0	5,805	3,895
Mean		64.6	1,010	861	1.42	1.58	6.55	6.90	4,183	3,553
SD		8.1	306	140	0.51	0.63	1.31	0.68	748	407
PE, 90% CI			1.12, 0.94–1.34						1.17, 1.09–1.25	
P			0.190						0.008	
Female subjects, n=6										
Mean		57.7	750	805	1.67	1.92	5.86	6.90	3,709	3,350
SD		3.7	267	165	0.61	0.74	1.14	0.80	301	302
Male subjects, n=6										
Mean		71.0	1,262	911	1.17	1.25	7.24	6.90	4,629	3,732
SD		3.7	180	82	0.26	0.27	1.16	0.62	799	425
P			0.020	0.174			0.038	1.00	0.046	0.093

Each capsule contained 100 mg CyA.
NIM Neoimmun, *SD* standard deviation, *PE* point estimate, *CI* confidence interval

after fasting administration evaluating data from 36 volunteers as well as after a fat-rich meal using 17 subjects. Nonetheless, after fasting administration, the 90% confidence interval was fully below 100% (AUC 90.6–98.0%, c_{max} 81.0–91.6%), and after a fat-rich meal, it was fully above 100% (AUC 100.8–108.3%, c_{max} 102.3–113.0%). As the bioavailability of Neoral is not increased after a fat-rich meal (Mueller et al. 1994), these results are in agreement with our present findings that the bioavailability of Neoimmun is significantly lower compared to Neoral when administered fasting and that the bioavailability increases significantly after a fat-rich meal.

Despite the significant differences between Equoral and Neoral, the results met—seen separately—the current bioequivalence criteria for approval as bioequivalent (Committee for Proprietary Medicinal Products 2002). Not surprisingly, the validity of the current standard criteria to establish bioequivalence between CyA formulations, based on studies in healthy volunteers, is the subject of some controversy (Castaneda-Hernandez et al. 1998; Cattaneo et al. 2005; Christians et al. 2000; Johnston et al. 2004; Meredith 2003; Qazi et al. 2006). However, pharmacokinetic studies in patients imply other drawbacks, such as the need of more subjects, due to the heterogeneity of the study

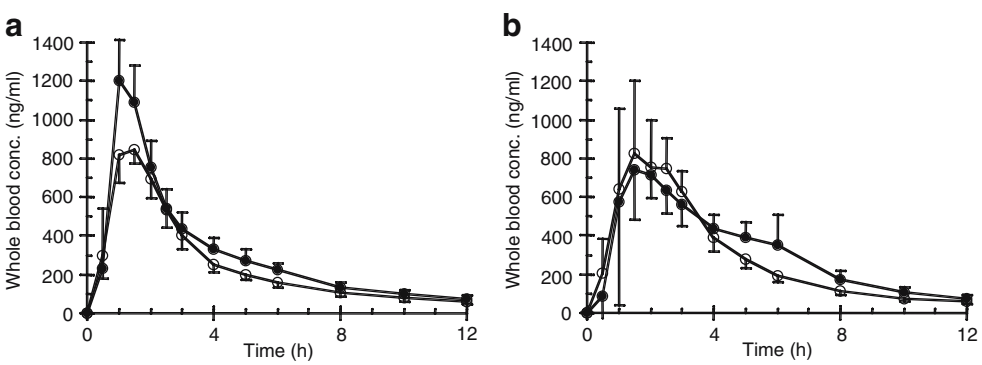

Fig. 2 Mean (SD) whole blood concentrations of CyA in six male (**a**) or six female (**b**) healthy young volunteers after oral administration of two capsules Neoimmun fasting (*open symbols*) or after a fat-rich breakfast (*closed symbols*). Each capsule contained 100 mg CyA

population or ethical problems if one group is possibly not adequately medicated. In a 4-week, multicenter, multinational pharmacokinetic study in 70 patients of different ethnic groups (30 Asian and 40 whites), bioequivalence has been concluded, as no significant differences were found between the pharmacokinetics of Neoral and Equoral (Masri et al. 2005). Nevertheless, significant differences in a subgroup of patients could have been ignored just because of the heterogeneity of the study population.

Based on the above-discussed issues, studies in well-defined groups of healthy subjects are indispensable in bioequivalence assessment procedures. However, we agree with the conclusion in a recent review on generic cyclosporine formulations (Cattaneo et al. 2005) "that more restrictive criteria are required to test generic formulations of narrow therapeutic index drugs,...as small variation in the pharmacokinetic property of generic formulation may result in a great impact on clinical outcome." At least, a retrospective analysis has revealed a significantly lower graft survival in renal transplant recipients given a generic CyA formulation compared with those on Neoral (cited in Cattaneo et al. 2005).

In our small, selected population of young healthy subjects, we observed a trend to an apparent lower bioavailability in female compared to male subjects indicating sex-related pharmacokinetics of CyA. The differences became more obvious when the bioavailability parameters c_{max} and AUC were normalized to 70 kg body weight. Albeit female subjects have a higher fat content, which could influence the tissue distribution of lipophilic drugs such as CyA, the normalization to body weight seems to be adequate, as studies in humans demonstrated comparable volumes of distribution in both obese and normal-weight individuals (Blouin and Warren 1999). Accordingly, CyA is generally dosed on a mg/kg base.

Several studies indicate that female rats clear CyA faster than males independently of the administration route or dosing regimen (Molpeceres et al. 2000). A literature survey revealed higher clearance in women than men for drugs that are CYP3A and *p*-glycoprotein substrates (Christians et al. 2006). Accordingly, we observed a trend to a lower bioavailability of CyA in female subjects during all treatments and periods. The differences were significant after a fat-rich meal, where the metabolic capacity of the body is more challenged than after fasting administration. These findings have not yet been confirmed in transplant patients. Either the relevant sex-related blood levels were not provided or the number of patients was too small. In a study population of 19 stable renal transplant recipients (9 males/10 females) using diltiazem as comedication to CyA (Aros et al. 2005), a known inhibitor of oxidative CyA metabolism, the mean increase during the comedication period was higher in male compared to female patients (c_{2h} 79 vs 4%, AUC 31 vs 13%) indicating a higher capacity of women to metabolize CyA. The differences were not significant, which should be due to the small number of subjects and the high variability of patients' demographic data, but the trend is in agreement with our results. Similarly, a study with young Japanese pediatric patients (32 boys and 20 girls aged 5 to 27 years) revealed no gender-related differences in the pharmacokinetics of CyA, but again, the study population was presumably too inhomogeneous to detect gender-related differences. However, the bioavailability increased with age in accordance with the observation that younger patients exhibit a higher rate of metabolism (del Mar Fernandez De Gatta et al. 2002; Dunn 2003). In this context, we ascribe our results rather to a higher metabolic capacity and therefore faster clearance in female subjects than to a decreased absorption. Furthermore, the trend to shorter half-life in female subjects is in agreement with this assumption. Unfortunately, we did not determine metabolites of CyA in this study.

At least, studies in experimental animals have provided evidence that there is a small but obvious sex-associated difference in the survival time of the graft (Hirashawa and Enosawa 1991; Hirashawa and Kamada 1992), with female recipients generally rejecting organ grafts in shorter time than males, which may be caused by sex-related differences in the pharmacokinetics of CyA.

Conclusion

In conclusion, the bioavailability of Neoimmun is significantly lower compared to Neoral when administered fasting in healthy volunteers. Because food intake has a variable impact on the bioavailability of Neoimmun, it should be administered on a consistent schedule in relation to meals. Future studies will have to determine whether the pharmacokinetic differences between Neoimmun and Neoral reported in this study are of clinical relevance in patients in terms of drug efficacy and side effects.

In addition, we suppose that the validity of the criteria based on studies in healthy volunteers could be improved if a more narrow range of bioequivalence than 80–125% were considered for CyA and/or if CyA medications were considered bioequivalent only if the 90% confidence interval were not only lying within certain limits but also included the value 100%. Those limits should be valid for fasting administration as well as after a meal.

As female and male subjects showed unexpected differences, i.e. at least the trend for a lower bioavailability of CyA in female subjects, we suggest that future studies in patients should include the analysis of blood concentrations related to sex and body weight to elucidate possible clinical implications.

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