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PHARMACOKINETICS OF CYCLOSPORINE G IN PATIENTS WITH RENAL FAILURE

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The pharmacokinetics of the cyclosporine A (CsA, Sandimmune) analogue Nva²-cyclosporine, or cyclosporine G (CsG) was investigated in 6 patients with terminal renal failure after a 4-hr intravenous infusion (3.5 mg/kg) and after oral administration (600 mg) of the drug. Blood samples were collected up to 38 hr and CsG concentrations were measured by radioimmunoassay and high-performance liquid chromatography. The resulting pharmacokinetic parameters of CsG were similar to those described for CsA in the same patient population. Based on HPLC determinations, a mean terminal elimination half-life of 18.9 hr was calculated. The total body clearance was 0.55 L/hr/kg, the volume of the central compartment was 0.32 L/kg, and the steady-state volume of distribution was 5.97 L/kg. After oral administration maximum CsG concentrations in blood were reached between 2.5 and 3 hr, and the bioavailability was in the range of 24–55% (mean 36%). The ratios between the polivalent RIA and HPLC determinations were considerably larger after oral dosing than after i.v. infusion. The blood-to-plasma ratio was 1.23, which is smaller than that observed for CsA. These results suggest that in patients undergoing renal transplantation the same dosing strategies can be applied for CsG as have been established for CsA.

Cyclosporine G (CsG),* or Nva²-cyclosporine is, like cyclosporine A (CsA, Sandimmun) produced by the fungus *Tolypocladium inflatum* GAMS (*1*). It differs from CsA by having a norvaline residue instead of alphaaminobutyric acid in position 2 of the molecule. In Wistar rats (*2*) and dogs (*3*) the immunosuppressive activity of CsG was found to be similar to that of CsA, but no nephrotoxicity was observed. On the other hand, in Sprague-Dawley rats the nephrotoxic and hepatotoxic side-effects were comparable to those observed with CsA (*4*), indicating that there are important differences between species and between animal strains. Therefore, carefully monitored clinical trials in man are indicated to confirm the lack of toxic side-effects that have been demonstrated at least in some animals. However, as a prerequisite for clinical investigations some knowledge of the relationship between dosage and CsG blood concentrations has first to be gathered. Thus, the aim of the present study was to investigate the pharmacokinetics of CsG after intravenous and oral administration in patients with terminal renal failure who were awaiting a renal transplantation and to compare the results with CsA.

MATERIALS AND METHODS

This investigation was designed as a randomized crossover study with a one-week washout period between the two CsG administrations.

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* Abbreviations used: AUC, area under the blood concentration curve; Cl, clearance; CsA, cyclosporine A; CsG, cyclosporine G, Nva²-cyclosporine; f, bioavailability; HPLC, high-performance liquid chromatography; RIA, radioimmunoassay; V_1, volume of the central compartment; Vdss, volume of distribution at steady-state.

To obtain some dosage guidelines for patients, a pilot study was carried out using the same protocol in healthy volunteers. For ethical reasons this group was restricted to two subjects. The protocol was approved by the Ethical Committee for Human Research of the Medical Department of the University Hospital and informed consent was obtained from all participants in the study.

The two healthy male volunteers were 19 and 20 years of age, and weighed 63 and 67 kg, respectively. The 6 patients were chronically hemodialyzed candidates for kidney transplantation with a mean age of 46 (34–59) years and a mean weight of 68 (51–89) kg. CsG was provided by Sandoz Ltd, Basel, Switzerland. The drug was infused via central venous line at a constant rate over 4 hr using a calibrated pump. All subjects received 3.5 mg/kg CsG, which was diluted in 500 ml glucose (5%). For the oral administration 600 mg CsG in 50 ml milk containing drinking chocolate (Caotina, Wander) was given after a 10 hr fasting period. A light breakfast was served after 2 hr and lunch 4 hr after drug administration.

Blood samples were drawn into ethylenediaminotetraacetate tubes at 0, 0.5, 1, 2, 3, and 4 hr during infusion, then 4.33, 4.66, 5, 5.5, 6, 7, 8, 10, 12, 14, 16, 22, 28, and 34 hr after the start of infusion. After oral dosing the sampling times were 0, 0.25, 0.5, 0.75, 1, 1.5, 2. 2.5, 3, 4, 6, 8, 10, 12, 15, 18, 24, and 30 hr after drug administration. All samples were split into two portions for RIA and HPLC analysis and stored at −20°C until assayed. In addition, 0.5 and 12 hr after the start of the infusion, and 3 and 12 hr after oral dosing, one aliquot of the blood samples was stored at room temperature (20–22°C) for 2 hr and centrifuged at 4000 rpm for 10 min to separate plasma. All samples (whole-blood and plasma) were analyzed by RIA and HPLC. The RIA method was performed with the commercially available RIA kit for CsA (*5*) using CsG as standards. The RIA detection limit was 22 ng/ml. In the concentration range from 100 to 2000 ng/ml the intraassay and interassay coefficients of variation (CV) were between 3.3 and 11% and between 4.2 and 11.3%, respectively. The HPLC method was similar to the technique described by Smith and Robinson (*6*), with the exception that an internal standard (CsA) was used and that the automated sample wash was replaced by a manual treatment with n-hexane. The interassay CV of the HPLC method for concentrations above 150 ng/ml was <10%, and the accuracy of spiked samples was found to be within 100±25% of the expected concentrations. The detection limit was 20 ng/ml. Regression analysis of 20 control samples measured by HPLC(x) and RIA(y) yielded in the equation y = 0.944 x +26.9 (r = 0.976).

Data analysis. Blood concentration-time curves after intravenous infusion measured by HPLC and RIA were fitted according to a three-compartment open-body model by the following equation:

$$C_b = \sum_{i=1}^{3} \frac{C_i(1 - e^{\lambda_i t'}) \cdot R_0}{-\lambda_i} \cdot e^{-\lambda_i t}$$

where C_i is the coefficient corresponding to a single intravenous bolus injection, R_o is the infusion rate, λ_i the exponential rate constant, and t' is the time from beginning of infusion (during infusion $t' = t$; thereafter t' becomes a constant equal to 4 hr). Data analysis was performed by the extended least-square fit program (ELSFIT, version 3.0) on an HP 9816 computer (*7*). All subsequently derived pharmacokinetic parameters were calculated using standard formulas (*8*).

After oral administration only model-independent pharmacokinetic parameters were calculated from blood concentration data of CsG. Bioavailability was calculated using the model independent area under the blood concentration curves (AUCs) from 0 to 30 hr. For calculation of the $AUC_{i.v.}$ the concentration at 30 hr was obtained by interpolation between 26 and 32 hr.

RESULTS

The pharmacokinetic results from the pilot study with two healthy volunteers were in the expected range and comparable with CsA. Peak CsG concentrations were 1264 ng/ml and 1558 ng/ml after i.v. infusion measured by HPLC and 712 ng/ml and 976 ng/ml after oral administration (Table 1). Reliable measurements were obtained up to 30 hr after oral administration and up to 32 hr and 38 hr after start of infusion using HPLC and RIA, respectively. The bioavailability was estimated at 21%. The elimination half-lives after i.v. infusion were rather short, being 6.7 and 3.7 hr, respectively. After these acceptable results, the study was continued with 6 patients using the identical protocol. Both routes of CsG administration were well tolerated by all patients except for a slight sensation of warmth

TABLE 1. Pharmacokinetic parameters of CsG after a single dose (3.5 mg/kg) administered as a 4-hr infusion in two healthy volunteers and in 6 patients with terminal renal failure

Patient	Dose (mg)	C_{max}[b] (ng/ml)	$t_{1/2_1}$ (hr)	$t_{1/2_2}$ (hr)	$t_{1/2_3}$ (hr)	k_{12}[c] (hr^{-1})	k_{22}[c] (hr^{-1})	k_{13}[c] (hr^{-1})	k_{31}[c] (hr^{-1})	Vd_{ss} (L/kg)	V_1 (L/kg)	Cl (L/hr/kg)	$AUC_{0-30\ hr}$[d] (ng/ml·hr)
CsG determined by HPLC:													
BP[a]	234	1264	0.044	0.70	6.7	8.85	2.81	2.51	0.230	2.26	0.150	0.414	9061
TD[a]	220	1558	0.072	0.87	3.7	4.47	1.65	1.31	0.303	1.34	0.167	0.497	7414
HE	273	3172	0.201	1.56	19.7	1.34	0.95	0.33	0.449	3.30	0.338	0.428	12282
WS	174	1024	0.067	0.55	27.5	2.66	1.80	3.04	0.045	11.60	0.166	0.651	4927
GJ	198	1168	0.148	1.62	22.9	1.81	0.78	0.97	0.051	9.17	0.411	0.445	7810
Bjo	217	1204	0.175	1.62	20.9	1.30	0.71	0.52	0.043	5.07	0.339	0.627	6638
SV	239	1704	0.119	1.20	14.0	2.25	1.08	0.67	0.064	2.41	0.179	0.427	8874
BJ	283	1610	0.112	1.43	8.4	3.15	1.25	0.69	0.126	4.25	0.474	0.739	9782
Mean ± SD[e]		1647	0.137	1.33	18.9	2.08	1.09	1.04	0.130	5.97	0.318	0.553	8385
		793	0.047	0.41	6.7	0.74	0.39	1.00	0.159	3.62	0.123	0.136	2558
CsG determined by RIA:													
Mean ± SD[e]		1891	0.112	1.93	26.2	4.74	0.998	0.715	0.098	6.14	0.247	0.434	10514
		624	0.048	0.55	13.1	4.94	0.223	0.316	0.135	4.28	0.113	0.096	3051

[a] Healthy volunteers.
[b] C_{max} at the end of infusion.
[c] k_{12}, k_{21}, k_{13}, and k_{31} are transfer rate constants between compartments.
[d] Interpolated between 26 hr and 32 hr.
[e] Patients only.

during the first hours of the trial. Representative blood concentration curves of one patient after intravenous infusion and oral administration are shown in Figures 1 and 2. Cyclosporine G concentrations measured by the polyvalent RIA were higher than those measured by HPLC in all patients. The mean ratios (±SD) between the two analytical methods are displayed in Figure 3. The considerably higher ratios after oral dosing indicate that there is a greater quantity of crossreacting metabolites after this route of cyclosporine G administration compared with i.v. infusion. The pharmacokinetic parameters after i.v. infusion are shown in Table 1. There is a large interindividual variability in most of the parameters. The terminal elimination half-life ($t_{1/2_\beta}$) measured by HPLC was 18.9 hr (range 8.4–27.5 hr). The volume of distribution at steady-state (Vd_{ss}) was 5.97 L/kg (range 2.41–11.6 L/kg), and total clearance (Cl) was 0.553 L/hr/kg (range 0.427–0.739 L/hr/kg). As expected, the pharmacokinetic parameters based on RIA determinations differed considerably from the HPLC derived data, with the most dominant difference for $t_{1/2_\beta}$ (mean value 26.2 hr; range 13.4–50.6 hr). Model-independent pharmacokinetic parameters after oral administration of CsG are shown in Table 2. Maximum CsG blood concentrations were reached between 2.5 and 3 hr, and the bioavailability (f) was 0.36 (range 0.24–0.55). A total of 24 plasma samples separated from whole blood at room

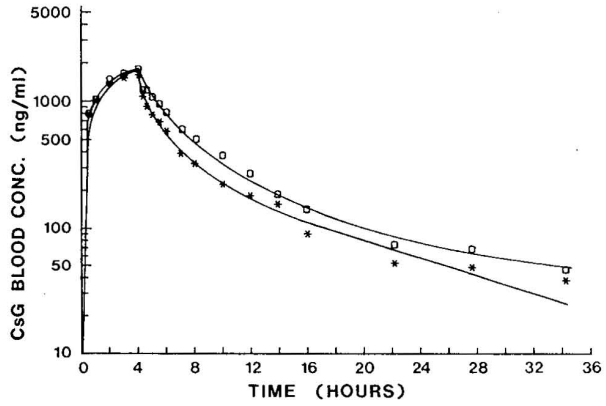

FIGURE 1. Cyclosporine G blood concentration in a patient (B.J.) after a 4-hr infusion (3.5 mg/kg) measured by RIA (○) and HPLC (★). The solid lines represent the computer fitted curves.

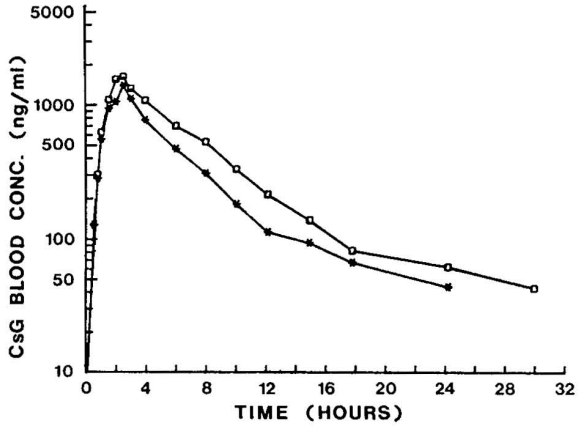

FIGURE 2. Cyclosporine G blood concentrations in a patient (B.J.) after oral administration (600 mg) measured by RIA (○) and HPLC (★).

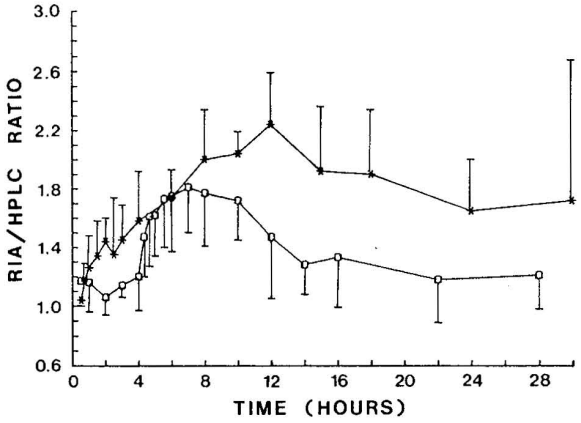

FIGURE 3. Ratios between CsG concentrations measured by RIA and HPLC after oral (★) and intravenous (○) dosing.

TABLE 2. Model independent pharmacokinetic parameters of CsG after a single oral dose (600 mg) in two healthy volunteers and in 6 patients with terminal renal failure

Patient	t_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-30\ hr}$ (ng/ml·hr)	f
CsG determined by HPLC:				
BP[a]	3.0	712	4994	0.215
TD[a]	3.0	976	4123	0.204
HE	2.5	1539	8067	0.299
WS	3.0	1134	6837	0.402
GJ	3.0	844	5570	0.235
BJo	2.5	1466	10120	0.551
SV	2.5	1362	7399	0.332
BJ	2.5	1392	7046	0.340
Mean ± SD[b]	2.67	1289	7506	0.360
	0.41	258	1521	0.108
GsG determined by RIA:				
Mean ± SD[b]	2.83	1791	12142	0.463
	0.26	196	2725	0.138

[a] Healthy volunteers.
[b] Patients only.

temperature were also measured by both methods. Mean blood: plasma ratios (±SD) were 1.23±0.18 (HPLC) and 1.23±0.23 (RIA).

DISCUSSION

In this study CsG was applied for the first time in man. Single doses of CsG were well tolerated by the patients and the volunteers of the pilot study. The pharmacokinetic parameters of CsG derived from patients with end-stage renal disease were similar to those found for CsA in the same patient population (*9*). After i.v. infusion a terminal average half-life ($t_{1/2_\beta}$) of 18.9 hr was calculated that is in good agreement with the half-life of 15.8 hr found for CsA. The values for the volume of distribution V_1 (0.318 L/kg) and for the total clearance Cl (0.553 L/hr/kg) are both larger than estimated for CsA (0.18 L/kg and 0.369 L/hr/kg). The pharmacokinetics of CsG after oral administration were calculated using a model-independent analysis. A bioavailability (f) of 36% (range 24–55%) was calculated that is in the same range as that reported for CsA (a review of the pharmacokinetic data of CsA) has been published elsewhere

[*10*]). For CsA a temperature-dependent uptake into blood cells has been observed (*11*), which is the main reason why it is recommended to measure this drug in whole blood rather than in serum or plasma. In the present study the concentration ratio between blood and plasma separated at 22°C was only 1.23, which is considerably lower than the ratio described for CsA. Similar to CsA all concentrations measured by RIA were higher than those measured by the specific HPLC. This signifies that the CsG metabolites crossreact also with the antiserum of the CsA-RIA used in this study. However, if the RIA:HPLC ratios between the two routes of administration are compared (Fig. 3) it is evident that these ratios are significantly ($P<0.01$) higher after p.o. administration than after i.v. infusion, which can be attributed to first-pass metabolism. A similar result was recently described for CsA in the dog (*12*), suggesting an additional metabolism in the gastrointestinal tract. Therefore, CsA or CsG blood concentrations measured by the polyvalent RIA should be interpreted with caution if the routes of drug administration are changed. It also means that determinations of blood cyclosporine concentrations by RIA to estimate the absolute bioavailability are not reliable.

In conclusion, the results of this study indicate that, in patients with renal failure, the pharmacokinetic behavior of CsG is similar to CsA—and, as a consequence, the same dosing strategies can be chosen until more clinical relevant data for CsG are available. In addition, clinical studies over longer periods are needed to confirm the optimistic results concerning reduced toxicity found in some animals.

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