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The kinetics of cyclosporine and its metabolites in bone marrow transplant patients

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1 The pharmacokinetics of cyclosporine (CsA) and the time course of CsA metabolites were studied in five bone marrow transplant patients after intravenous (i.v.) administration on two separate occasions and once after oral CsA administration.
2 Cyclosporine and cyclosporine metabolites were measured in whole blood by h.p.l.c.
3 Cyclosporine clearance after i.v. administration decreased from 3.9 ± 1.7 ml min^{-1} kg^{-1} to 2.0 ± 0.6 ml min^{-1} kg^{-1} after 14 days of treatment. The mean ± s.d. absolute oral bioavailability of cyclosporine was 17 ± 11%.
4 Hydroxylated CsA (M-17) was the major metabolite in blood. There were no significant differences in the mean metabolite/CsA AUC ratios between the first and second i.v. studies.
5 After oral administration, the metabolite to CsA AUC ratios were higher for most metabolites compared to those observed in the second i.v. study, suggesting a contribution of intestinal metabolism to the clearance of CsA.

Keywords cyclosporine pharmacokinetics drug metabolism drug analysis bone marrow transplantation

Introduction

Cyclosporine (CsA) is an immunosuppressant used to prevent rejection of transplanted organs and to prevent or treat graft-versus-host disease in patients undergoing allogeneic bone marrow transplantation (BMT). The kinetics of CsA have been reported to be highly variable among and within patients over a given time period (Venkataramanan *et al.*, 1989). An increase in the AUC of CsA with chronic therapy has also been observed and attributed to improvement in oral bioavailability (Kahan *et al.*, 1983) or alterations in the distribution and binding of CsA and its metabolites in blood (Awni *et al.*, 1989). Auto-inhibition of CsA metabolism could also account for an increased AUC of CsA with long-term therapy (Habucky *et al.*, 1988, 1990).

CsA is metabolized primarily by the hepatic mixed function oxidase system with subsequent excretion of metabolites into bile and urine (Burckart *et al.*, 1986; Maurer, 1985). As many as 27 distinct metabolites have been identified in human blood, urine, or bile (Maurer & Lemaire, 1986; Wallemacq *et al.*, 1989). Recent reports indicate that some CsA metabolites (M-17 and M-1) may possess immunosuppressive activity as measured by *in vitro* inhibition of lymphocyte proliferation (Burckart *et al.*, 1988; Freed *et al.*, 1987; Rosano *et al.*, 1986 a; Zeevi *et al.*, 1988 a,b). Thus, the metabolites may play an important role in the clinical immunosuppression resulting from CsA therapy. There is little information available on the time course of formation and elimination of CsA metabolites in humans.

The contribution of metabolites to the toxicity of CsA is also a matter of considerable debate. Some authors have suggested that they may contribute to CsA-induced nephrotoxicity (Yee *et al.*, 1986), whereas others have implicated the parent compound in the production of these effects (Cole *et al.*, 1989; Cunningham *et al.*, 1983). A knowledge of the concentrations of CsA metabolites relative to the concentration of the parent compound may help to elucidate their contribution to immunosuppressant activity *in vivo* as well as their potential for toxicity.

The objectives of this study were: 1) to compare the intravenous (i.v.) pharmacokinetics of CsA within 1 week of bone marrow transplantation (BMT) with those measured 2 or 3 weeks after BMT; 2) to calculate the

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oral bioavailability of CsA in BMT patients; 3) to compare the concentration-time profiles of four metabolites (M-17, M-1, M-18, M-21) within 1 week of BMT with those observed 2 to 3 weeks later; and 4) to compare the metabolic profile after i.v. and oral therapy.

Methods

Patients

The study was approved by the Protection of Human Subjects Committee of Montefiore University Hospital, and written informed consent was obtained from each patient. Five men (median age 33, range 16–38 years) undergoing allogeneic BMT for the treatment of haematological malignancy were enrolled in the study. The conditioning regimen consisted of high-dose busulfan and cyclophosphamide (patients 1–4) or high-dose cyclophosphamide and total body irradiation (patient 5) as described previously (Rosenfeld *et al.*, 1989). Patients received 5 days of phenytoin therapy (300 mg daily) concurrently with busulfan for seizure prophylaxis. Bone marrow from a human lymphocyte antigen-matched donor was infused 2 days after completion of the conditioning regimen in those who received busulfan/cyclophosphamide and 1 day after regimen completion in the patient who received cyclophosphamide/total body irradiation. The regimen for prevention of graft-versus-host disease consisted of i.v. CsA (beginning one day prior to marrow infusion) and i.v. methylprednisolone (beginning 7 days after marrow infusion). Oral CsA and prednisone were initiated several days prior to hospital discharge. Patients had relatively normal and stable serum creatinine (range 0.6–1.4 mg 100 ml^{-1}), total bilirubin (range 0.6–1.6 mg 100 ml^{-1}), and total cholesterol (range 98–179 mg 100 ml^{-1}) values throughout the study period. Packed red blood cells and platelet transfusions were given as required to maintain haematocrit and platelet counts at acceptable levels. There were no significant changes in laboratory parameters among study periods.

CsA administration and blood sampling

CsA (1.5 mg kg^{-1}) was mixed in 5% w/v dextrose solution and infused i.v. over 4 h, twice daily beginning 1 day prior to marrow infusion. Intravenous therapy was continued for 16 to 22 days, depending upon the patient's condition and ability to take oral medications. Oral therapy was instituted thereafter at approximately three times the current i.v. dose. When given orally, CsA was mixed in chocolate milk or another palatable vehicle and taken twice daily. Serial blood samples were collected for the pharmacokinetic study from all five patients on i.v. infusion within 3 to 7 days of BMT and on a second occasion 14 to 20 days after BMT. Four of the patients were studied a third time (18 to 27 days after BMT) after receiving at least 3 days of oral CsA at 2.9 to 4.2 times the i.v. dose. For the i.v. studies, samples were obtained immediately prior to the infusion (zero hour), during the midpoint of the infusion (2 h), and sequentially after completion of the infusion (4, 4.5, 5, 6, 7, 8, 9, 10, and 12 h after the infusion was begun). For the oral dosage studies, samples were obtained immediately prior to the dose (0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h thereafter.

Measurement of CsA and metabolites

CsA and metabolite concentrations were assayed in whole blood by the gradient elution h.p.l.c. method described by Wang *et al.* (1988 a). Briefly, 2 ml whole blood were mixed with an internal standard (cyclosporine D) and extracted into diethyl ether. After washing with hexane, the final dried extract was reconstituted in 100 μl methanol, and 40 μl was injected onto a Resolve C-18, 15 cm × 3.9 mm, 5μ column (Waters; Milford, MA) that was maintained at 70° C. The mobile phase consisted of a linear gradient of acetonitrile and water delivered at a flow rate of 1.0 ml min^{-1}. Standards of the metabolites were prepared from material recovered from human bile and confirmed by h.p.l.c. and mass spectrometry. Standard curves were prepared for CsA and each of the four metabolites that were measured. The minimum measurable concentration of CsA and metabolites was 20 ng ml^{-1}. The standard curve was linear over a concentration range of 20 to 2,000 ng ml^{-1}. The coefficients of variation for CsA and metabolite concentration measurements were 1.8% for M-17, 4.9% for M-1, 9.3% for M-18, 7.6% for M-21, and 1.8% for CsA.

Pharmacokinetic and statistical analyses

Confirmation of steady state was established by comparing concentrations at time zero (prior to the dose) and at the end of the dosing interval (12 h). These CsA concentrations were within ± 15% of each other (range −15% to +12%). Patients received 3 or more days of CsA treatment prior to the first study period. The areas under the plasma concentration *vs* time curves (AUCs) from time zero to the next dose for CsA and metabolites over each dosing interval at steady-state were calculated using the linear trapezoidal rule. The AUC values were normalized for dose. The ratio of the AUC of each metabolite to the AUC of CsA was calculated during each dosage interval studied. The percent oral bioavailability of CsA relative to the second i.v. study period was calculated from: $(AUC_{po}/DOSE_{po} \times DOSE_{iv}/AUC_{iv}) \times 100$. CsA clearance was calculated from DOSE/AUC(T) at steady state.

Data are reported as mean ± s.d. where appropriate. Statistical comparison of CsA clearance, dose-normalized AUCs, and CsA-to-metabolite AUC ratios during the three study phases was performed by analysis of variance. Pairwise comparisons were analyzed by Duncan's multiple range test. A value of $P < 0.05$ was considered to be significant. All statistical analyses were performed using the Statistical Analysis System (SAS Institute, 1985).

Results

Cyclosporine concentration increased substantially during the 4 h i.v. infusion with a mean peak of 3200 ± 1769 ng ml^{-1}. The CsA concentration then declined

rapidly to a mean concentration of 518 ± 333 ng ml^{-1} within 4 h after the end of infusion. The concentration declined slowly from the fourth to the eighth hours after the completion of the infusion, reaching a mean trough level (C_{min} at steady state) of 402 ± 252 ng ml^{-1}. Administration of CsA every 12 h precluded calculation of an accurate disposition rate constant and disposition half-life. The mean clearance during the first i.v. study was 3.9 ± 1.7 ml min^{-1} kg^{-1}.

Comparison of i.v. CsA pharmacokinetics between periods

The mean dose-corrected CsA AUC increased from 71 ± 35 ng ml^{-1} h kg^{-1} during the first i.v. period to 123 ± 37 ng ml^{-1} h kg^{-1} during the second i.v. period, but this trend was not statistically significant ($P = 0.08$, Table I). Correspondingly, CsA clearance tended to decrease from the first to the second i.v. period (from 3.9 ± 1.7 ml min^{-1} kg^{-1} to 2.0 ± 0.6 ml min^{-1} kg^{-1}; $P = 0.06$).

Oral CsA pharmacokinetics

The mean peak CsA concentration was 934 ± 695 ng ml^{-1}, and the mean time to the peak concentration was 3.7 h (range 0.5–8.0 h). The mean minimum concentration of CsA at the end of the study interval was 304 ± 126 ng ml^{-1}. The mean oral bioavailability of CsA relative to the second i.v. study period was 17 ± 11%.

Metabolite profile

Figure 1 shows a typical metabolic profile in one patient. The hydroxylated metabolite M-17 was the major meta-

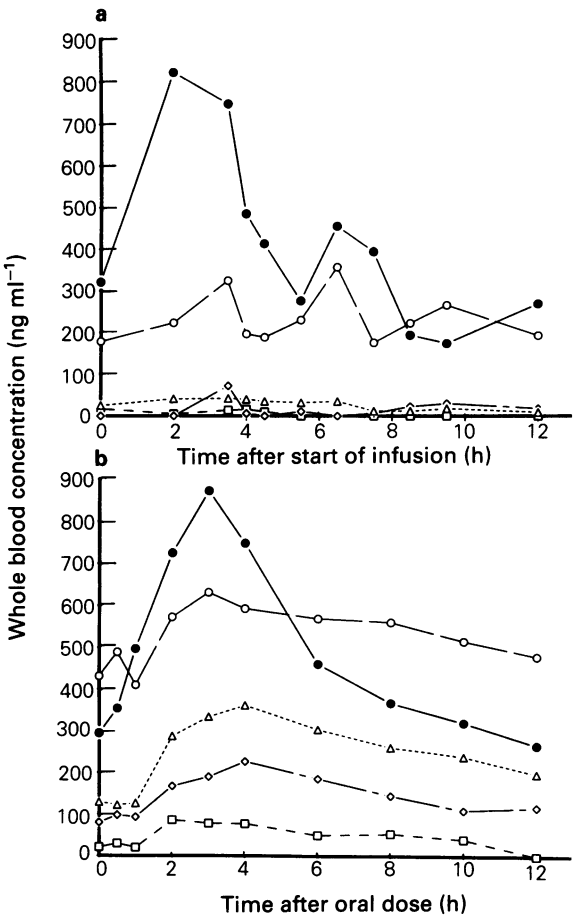

Figure 1 CsA (●), M-17 (○), M-1 (△), M-18 (◇), and M-21 (□) concentrations in whole blood over a 12-h dosing interval during intravenous (a) and oral (b) therapy in a marrow transplant patient.

Table 1 Dose-corrected AUC of CSA and metabolites for each study phase [ng ml^{-1} h)/dose]

Patient	*Period*	*Dose (mg)*	*CsA*	*M-17*	*M-1*	*M-18*	*M-21*
1	1st i.v.	115	45	17	0	1	0
	2nd i.v.	115	114	30	1	3	0
	Oral	480	8	9	3	1	1
2	1st i.v.	140	35	19	2	1	0
	2nd i.v.	140	79	38	3	6	0
	Oral	400	15	16	8	5	2
3	1st i.v.	105	129	29	3	3	0
	2nd i.v.	80	179	56	4	2	1
	Oral	250	18	15	8	0	0
4	1st i.v.	91	94	45	2	5	0
	2nd i.v.	91	149	85	10	13	0
	Oral	300	47	43	15	12	1
5	1st i.v.	139	52	24	0	6	0
	2nd i.v.	139	92	41	1	5	0
Mean	1st i.v.		71	27	1	3	0
	(± s.d.)		(35)	(10)	(1)	(2)	(0)
	2nd i.v.		123	50	4	6	0
			(37)	(19)	(3)	(4)	(0)
	Oral		22	21	9	4	1
			(15)	(13)	(4)	(5)	(1)

bolite in whole blood. In contrast to the wide fluctuation in cyclosporine concentrations over the dosing interval, those of M-17 remained within a relatively narrow range throughout the 12 h study period after i.v. dosing, with mean peak and trough concentrations of 486 ± 219 and 302 ± 110 ng ml^{-1}, respectively. At the twelfth hour, the M-17 concentrations exceeded those of CsA concentrations in four of ten i.v. cases. The concentrations of M-1, M-18, and M-21 were much lower than those of CsA or M-17 during the entire 12 h. The pattern of metabolite production did not change from the first to the second i.v. study period.

Variability of AUCs among patients

Table 1 lists the dose-corrected AUCs of CsA and the metabolites for each of the patients during each study phase. There were significant differences among the patients in the AUCs of CsA, M-17, M-1, and M-18. The interpatient variability in the M-21 AUC was not significant, but the absolute AUCs were very small.

After i.v. administration, the mean dose-corrected CsA AUC was greatest (Table 1), the M-17 AUC intermediate, and the AUCs of M-1, M-18, and M-21 the least. As with cyclosporine, the mean M-17 AUC tended to increase with time, but the AUCs of the other metabolites did not.

Comparison of metabolite-to-CsA AUC ratios after i.v. dosing

The ratio of the AUC of each metabolite to the AUC of CsA in each period is shown in Figure 2. In both i.v. periods, the M-17 ratio was larger than that of the other metabolites. There were no apparent differences in the AUC ratio between the first and second i.v. periods for any of the four metabolites. There were no significant differences in the metabolite-to-CsA ratios among patients.

Metabolic profile after oral therapy

In all patients M-17 concentrations were higher after oral than after i.v. dosing; at the end of the dosing interval the M-17 concentration exceeded that of CsA in all four patients studied after an oral dose. The M-1 and M-18 levels paralleled the M-17 profile, but the absolute concentrations were lower. There were low but detectable concentrations of M-21 over most of the oral dosing interval.

After oral administration the metabolite-to-CsA AUC ratios increased compared with the second i.v. phase (Figure 2). The M-17 to CSA ratio was approximately unity after oral dosing. The ratios of M-1, M-18, and M-21 were less than 0.2 during each period with the exception of M-1, which increased to 0.43 during the oral period.

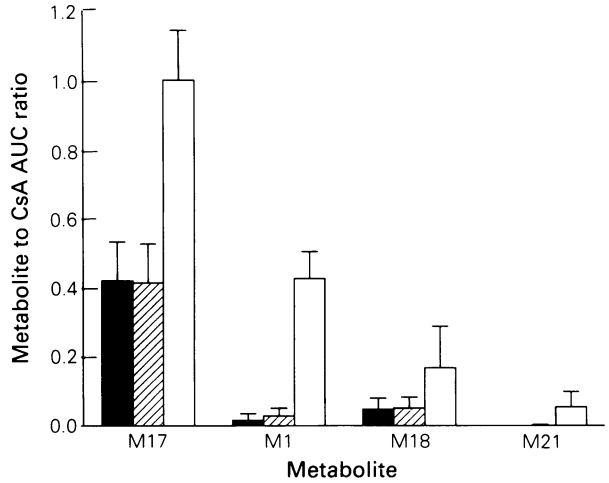

Figure 2 Mean (± s.d.) ratio of AUC of metabolite to AUC of CsA during the first i.v. (■), second i.v. (▨), and oral (□) study phases.

Discussion

The mean clearance of CsA in this study indicates this drug to be a low clearance drug in marrow transplant patients. This is consistent with the findings in patients with solid organ transplants (Venkataramanan *et al.*, 1989). There was a large variability in CsA clearance between patients. While the steady state clearance of CsA during the first week after marrow transplantation was similar to values observed in adult solid organ transplant patients (Ptachcinski *et al.*, 1986), the clearance during the second or third week was lower. Differences in the clearance of CsA were also seen within individual marrow patients between the two study periods. Unlike those receiving solid organ transplants, marrow transplant recipients receive a marrow ablative conditioning regimen prior to transplantation. This regimen may affect the clearance of CsA. Induction of the hepatic mixed function oxidase system by the phenytoin given for 5 days for seizure prevention while patients received high-dose busulfan may have led to a transient increase in drug clearance early after BMT without changes in the results of liver function tests (Keown *et al.*, 1984). However, in a prospective study of CsA for prevention of graft-versus-host disease, we found no difference between the patterns of changes of trough CsA concentrations in patients conditioned with busulfan and cyclophosphamide as compared with cyclophosphamide and total body irradiation (Przepiorka *et al.*, unpublished data).

The overall CsA metabolite profile in BMT patients is similar to that previously reported in other transplant patients (Awni *et al.*, 1989; Rosano *et al.*, 1986 b; Wang *et al.*, 1988 a,b). The metabolite concentrations tended to decline in parallel to those of CsA. This is similar to what has been observed in liver transplant patients and in dogs (unpublished observation; Habucky *et al.*, 1989), indicating that the disposition of the metabolites is formation-rate limited. There was significant variability among BMT patients in the dose-corrected AUCs of all metabolites except M-21, which was present in the lowest concentrations and usually only after oral therapy. The high M-17 AUC relative to other metabolites and the high M-17 trough concentrations relative to CsA are particularly noteworthy given the high *in vitro* immunosuppressive activity of M-17 (Burckart *et al.*, 1988; Freed *et al.*, 1987; Rosano *et al.*, 1986a; Zeevi *et al.*, 1988 a,b). Trough M-17 concentrations that approach or exceed

those of CsA have also been observed in liver, heart, and renal transplant patients (Rosano *et al.*, 1986 b; Wang *et al.*, 1988 a,b). In the present study, the AUCs of CsA and its metabolites increased from the first to the second i.v. study period, but the metabolite-to-CsA ratios remained constant. These results are similar to those reported by Awni *et al.* (1989) in a group of renal transplant patients. These investigators showed that the dose-normalized CsA AUC increased over the first 12 weeks after transplantation and initiation of CsA therapy. In that study, factors that may alter the binding and distribution of CsA (haematocrit, plasma proteins, and lipoproteins) also showed a significant rise over the 12-week study period, and the rise correlated with the change in CsA AUC. Because of these findings and because the metabolite-to-CsA ratios did not change over the study period, the authors concluded that alterations in CsA distribution and binding, rather than an improvement in bioavailability or a reduction in CsA metabolism, were likely to be responsible for the change in CsA kinetics over time.

In contrast to the findings of Awni *et al.* (1989), we observed an increase in the CsA AUC within the first three weeks of therapy. Because of the relatively short time interval between study periods, there was no significant change in cholesterol levels between study periods. Because of the intermittent infusion of packed red blood cells, there was also no marked change in haematocrit from the first to the second i.v. study periods. Thus, the reasons for the observed increase in AUC over time in the present study are not likely to be related to altered CsA binding with time. All patients had received 3 or more days of CsA prior to the first study period, which allowed sufficient time for CsA to achieve steady-state concentrations. Changes in hepatic or renal function are unlikely to account for the rise in AUC over time because only inconsequential, transient changes in hepatic or renal function tests occurred.

Another possible explanation for the increased CsA AUC over the first 2–3 weeks of therapy is the development of CsA-induced inhibition of hepatic metabolism. Augustine & Zemaitis (1986) demonstrated that cyclosporine inhibits the hepatic mixed function oxidase system in rats after 9 days of daily administration. Habucky *et al.* (1988) observed a significant increase in CsA half-life in dogs after pretreatment with CsA. Although a decrease in the metabolite-to-CsA ratio might be expected as a result of reduced metabolite formation, the ratio could remain relatively constant if the elimination of metabolites was also impeded.

After oral administration CsA was absorbed fairly rapidly, but the extent of absorption was incomplete. The oral bioavailability of CsA varied four-fold and the mean bioavailability was lower in marrow transplant patients (17 ± 11%) than that previously reported in solid organ transplant patients (approximately 30%) (Venkataramanan *et al.*, 1989). This may be related to the functional integrity of the gastrointestinal mucosa in these patients. Gastrointestinal dysfunction is commonly observed in marrow transplant patients and may reduce blood CsA concentrations after oral dosing (Atkinson *et al.*, 1984).

A substantial increase in the AUC of the metabolites and the metabolite-to-CsA ratios occurred after oral dosing. The ratio of the dose-corrected AUC of the metabolite after oral dosing to that after i.v. administration is greater than 1.8. Since CsA is not excreted by the kidney to any significant extent, this indicates possible intestinal metabolism of CsA (Pang *et al.*, 1981). This observation may have important implications for chronic oral CsA therapy. Low blood concentrations of CsA may provide satisfactory immunosuppression if concomitant high concentrations of active metabolites are present.

High concentrations of cyclosporine metabolites are present in the blood of BMT patients, especially after oral dosing. The interindividual variability in the production of these active metabolites may partially explain differences in immunologic response among patients to a given CsA dose. The potential immunosuppressive effects of CsA metabolites must be considered in future studies of the effects of CsA in BMT patients. Because of the variable relationship between CsA and metabolite concentrations, monitoring CsA concentrations alone may not be predictive of the total immunosuppressive activity of the drug.

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