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Long-term cyclosporin A pharmacokinetic profiles in pediatric renal transplant recipients

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Abstract To study the long-term effect of cyclosporin A (CyA), 94 6-h and 29 12-h pharmacokinetic profiles were evaluated in 32 children at least 1 year after renal transplantation. Children weighing less than 25 kg needed significantly higher doses of CyA than those weighing more than 25 kg(9.8 vs 5.3 mg/kg per day; $P < 0.001$) to achieve similar trough levels (TL). The average dose of CyA required to achieve the target TL declined gradually with time after transplantation. The average area under the curve over 6 h (AUC/6) correlated strongly with the AUC/12 ($r = 0.967$; $P < 0.001$). The AUC/6 of patients with biopsy-proven CyA toxicity was significantly higher than for those without toxicity (Mann-Whitney U-test $P < 0.05$) despite similar TL. We conclude that AUC monitoring for 6 h provides valuable information not only on TL but also on the absorption and elimination characteristics of CyA as well as on the potential for CyA toxicity.

Key words Cyclosporin A, renal transplantation, children · Renal transplantation, children, CyA · Pediatric, CyA, renal transplantation · Pharmacokinetics, children, CyA

Introduction

Cyclosporin A (CyA) is a selective immunosuppressive agent that has been widely used in pediatric organ transplantation during the past decade [2]. By monitoring CyA trough levels (TL) and adjusting the CyA dose, it is expected that dose-dependent nephrotoxicity as well as allograft rejection can be reduced [5, 7, 15, 16, 22]. A TL is supposed to be representative of the corresponding total blood concentration time curve, yet this assumption has never been proved for CyA. Grevel et al. [6] showed that the area under the blood concentration time curve (AUC) is superior to TL both in its correlation with the oral dose and in reducing the frequency of dosage adjustments. Abbreviated AUC monitoring has already been introduced in adult practice [16]. Since long-term experience with CyA in the pediatric transplant population is small, we analyzed dosage concentration-nephrotoxicity effect relationships retrospectively, based on abbreviated pharmacokinetic profiles of pediatric kidney transplant recipients.

Patients and methods

Between 1989 and 1992, 32 consecutive, clinically stable, renal transplant recipients were studied at least 1 year after transplantation. The patients included 20 boys and 12 girls with a mean age of 9.5 years (range 15 months to 18 years). All patients gave their informed consent. Nine grafts were from living related donors and 23 grafts were cadaveric. For 28 patients it was the first transplant and for 4 the second. The mean follow-up period was 3.8 ± 2 years (range 1–12 years).

Immunosuppressive treatment consisted of a triple regimen with prednisolone (0.1–0.3 mg/kg per day p.o.), CyA, and azathioprine (1–1.5 mg/kg per day p.o.). CyA medication was taken twice daily, dispersed in orange juice or chocolate milk. Each intake was followed by a meal. No patients changed dispersion medium dur-

Table 1 Long-term parameters of the patients (mean ± SD and range)

Time after Tx	No. of patients	Creatinine (mg/dl)	CyA (mg/kg per day)	TL (ng/ml)
1 (years)	31	0.99 ± 0.36	8.2 ± 2.5	98 ± 25 (49–158)
2 (years)	26	1.04 ± 0.34	7.1 ± 3.0	97 ± 36 (37–212)
3 (years)	23	1.15 ± 0.40	5.5 ± 2.1	92 ± 52 (25–239)
4 (years)	14	1.09 ± 0.25	4.5 ± 1.6	92 ± 42 (26–178)
≥ 5 (years)	17	1.26 ± 0.34	5.1 ± 2.3	90 ± 52 (29–261)

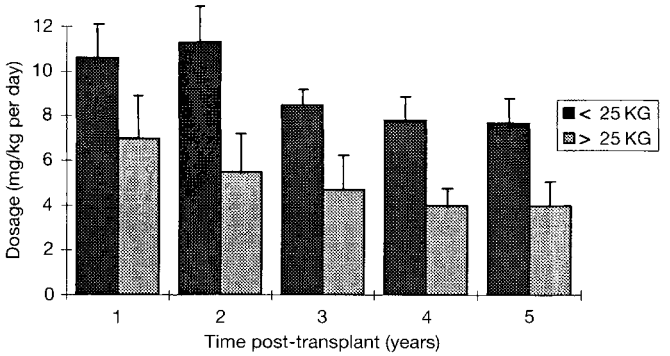

Fig. 1 Mean CyA daily dosage in relation to body weight

ing the follow-up period. In two children, azathioprine was discontinued during the follow-up period because of bone marrow toxicity. No patients used any drugs, such as diltiazem, verapamil, or erythromycin, that might interfere with the pharmacokinetics of CyA. In one patient, CyA was started 10 years after transplantation in order to lower the doses of the other immunosuppressive agents in relation to the presence of hepatitis C infection.

All patients were evaluated on an outpatient basis at regular intervals after transplantation. During the 1st year after transplantation, it was every 2 weeks, and later on the follow-up period varied between 3 weeks and 2 months. Each clinical check-up consisted of a physical examination and laboratory investigations including peripheral blood analysis for serum creatinine and morning 12-h TL of CyA. All CyA determinations were measured in whole blood by selective radioimmunoassay (Cyclo-Trac SP kit, Incstar, Stillwater, MN, USA) [25]. The tracer was ^{125}I-labeled CyA. This procedure uses a monoclonal antibody that selectively recognizes the parent drug but not its metabolites. Whole blood standards from 20 to 1200 μg/l, supplied by the manufacturer, were used in these RIA measurements. Within-assay and between-assay coefficients of variation for concentrations in the range of 50–800 μg/l were lower than 10% [23]. The lower detection limit for the assay was 25 ng/ml. CyA dosage was regularly and individually adjusted to maintain a target 12-h whole blood TL within the range of 75–175 ng/ml.

From 1989 to 1992, 94 6-h and 12-h CyA pharmacokinetic profiles of the transplant recipients were established during a yearly check-up. For the 94 6-h profiles, whole blood samples were drawn by venipuncture just before and at 1, 2, 4, and 6 h after the morning oral dose. Additional blood samples were taken for 29 12-h profiles at 8, 10, and 12 h after the oral intake.

In 14 patients, a sonographically guided kidney biopsy was performed with an automatic biopsy device (14 G needle) in order to elucidate the cause of elevated serum creatinine. Criteria for CyA toxicity on renal biopsy included striped atrophy of tubules, hyalinosis of the arteriolar media, and diffuse interstitial fibrosis [18]. All specimens were re-examined blindly for the purpose of this study.

The area under the curve (AUC) of the whole blood concentration versus time curve, which is a summation of the entire profile, was calculated by use of the linear trapezoidal rule. Each AUC was divided by the time of dose interval (AUC/t) to obtain the average concentration at steady state.

For statistical evaluations, the Mann-Whitney U-test and linear regression analysis were used. Statistical significance was defined as a P level below 0.05.

Results

During the 120 patient years after transplantation, 17 acute rejection episodes occurred in 14 of the 32 children. They all occurred in the 1st year after transplantation, before the start of this study. Table 1 shows the parameters (mean ± SD) of the patients during the long-term follow-up: serum creatinine, CyA doses, and TL at 1, 2, 3, 4, and 5 or more years after transplantation.

Mean serum creatinine remained stable over the follow-up period after transplantation. At the end of the 1st year, only 31 patients were taking CyA, and in one patient CyA was started 10 years after transplantation. The mean (± SD) CyA oral dose required to achieve a constant 12-h CyA TL declined gradually after transplantation from 8.2 ± 2.5 to 4.5 ± 1.6 mg/kg per day after 4 years.

After subdividing the population into two groups – one weighing more and one weighing less than 25 kg – we found that the smaller children required significantly higher doses of CyA (9.8 ± 1.7 mg/kg per day) than the larger ones (5.3 ± 1.9 mg/kg per day; Mann-Whitney U-test $P < 0.001$). The difference remained significant during the entire follow-up period (Fig. 1). Trough levels of the two groups did not differ significantly.

For the observed time interval, a linear relationship was found between the doses of CyA in mg/kg and the doses in mg/m^2. (CyA in mg/m^2 body surface) = 53.6 + 20.3 × (CyA in mg/kg body weight), with $r = 0.95$ and $P < 0.001$ (Fig. 2). The dosage calculated per body surface was also significantly higher for smaller children (238 ± 44 mg/m^2) than for larger ones (164 ± 55 mg/m^2; Mann-Whitney U-test $P < 0.001$). This difference remained significant during the entire follow-up period.

Three different types of concentration versus time profiles of CyA were found in children: (1) a single early

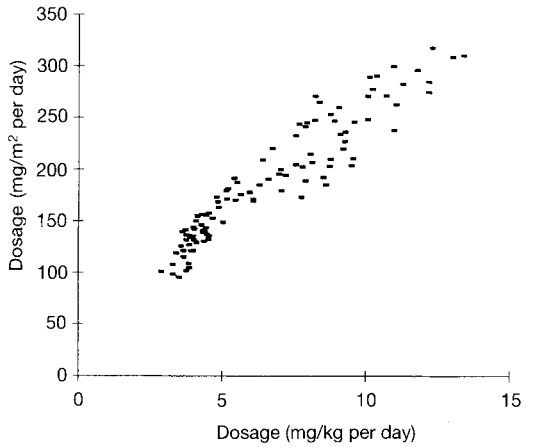

Fig. 2 Mean CyA daily dosage in mg/kg versus mg/m^2

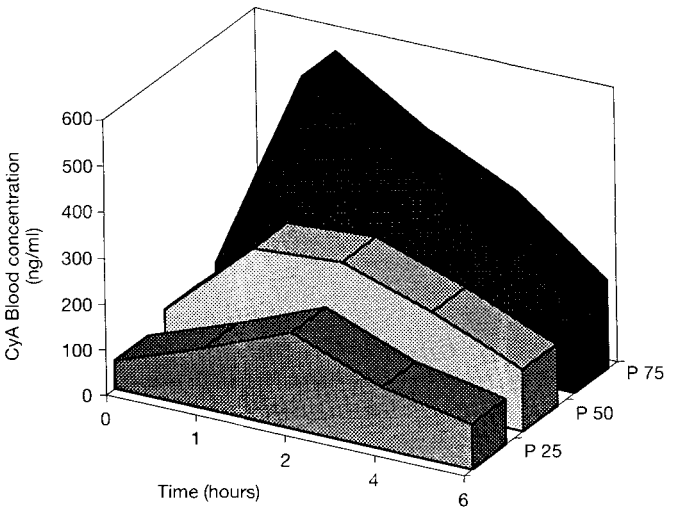

Fig. 3 Overview of 94 6-h CyA concentration profiles

peak after oral administration, (2) a biphasic peak, and (3) a sustained, flattened curve with little concentration increment.

The 94 pharmacokinetic profiles (Fig. 3) showed median (P25-P75) CyA levels (in ng/ml) of: 92 (62–111) before morning dose administration, 269 (133–561) after 1 h, 283 (212–434) after 2 h, 217 (134–342) after 4 h, 135 (97–193) after 6 h, 124 (83–161) after 8 h and 92 (74–120) after 12 h (8-h and 12-h data not shown).

Table 2 shows the results of maximum concentration (C_{max}), the steady state concentration (AUC/6), and the TL in the 94 pharmacokinetic profiles over the follow-up period. The mean values of the CyA TL 1 year after transplantation are a little higher than the later values. This is due to our policy of keeping the TL near the maximum of the target range during the 1st year post-transplantation in order to minimize the incidence of rejections.

The AUC/12 in the 12-h profiles correlates strongly with the AUC/6 values used in the 6 h profiles ($r = 0.97$; $P < 0.001$). We found no correlation between the AUC/6 and the serum creatinine concentration ($r = 0.09$; P = NS). The absorption of CyA in children is rapid: for 75 % of the patients, a maximum concentration of 472 ± 262 ng/ml was reached within 2 h (T_{max}) after oral administration of the drug.

We further analyzed whether TL or average steady state concentrations were the most appropriate measure for CyA toxicity. Of three children with CyA toxicity on biopsy, only one had a TL of 212 ng/ml, which was higher than the target TL. The TL of the other children were in the normal range. There was no statistical difference in TL of CyA or in serum creatinine levels between 3 patients with biopsy-proven toxicity and the 11 patients with no evidence of CyA toxicity on biopsy. The mean steady state average concentration (± SD) from the AUC/6 values of patients with CyA toxicity on biopsy (479 ± 67 ng/ml) was significantly higher than that of those without toxicity (219 ± 99 ng/ml, $P < 0.05$; Table 3). In none of the children without toxicity was the AUC/6 above 400 ng/ml.

Discussion

In renal transplantation, optimal dosing and monitoring of CyA is essential for successful graft outcome. Despite extensive studies of CyA pharmacological behavior, there is still a therapeutic uncertainty due to the fact that CyA not only has a immunosuppressive effect but also a nephrotoxic one. It was shown that AUC is superior to TL both in its correlation with the oral dose [13] and in reducing the frequency of dosage adjustments [6]. According to Grevel et al. [6] average concentrations at steady state are better indicators of the clinical efficacy and safety of CyA in adults.

In children especially, pharmacokinetic profiles are necessary to discover absorption problems or altered CyA clearances that require dosing adjustments [11, 17, 20, 21], not only in the 1st months after transplantation but also after long-term use of CyA, since CyA toxicity remains a major concern. We therefore retrospectively analyzed pharmacokinetic data from 32 children more than 1 year after kidney transplantation. As already demonstrated [8], the oral dose of CyA that our patients required to achieve similar CyA morning trough levels declined gradually with time after transplantation, possibly due to an improved drug bioavailability observed with chronic administration [12]. Awni et al. [1] reported that changes in CyA pharmacokinetics over time are due to alterations in the distribution and binding of CyA and its metabolites in blood, rather than to a reduction in the metabolism of CyA or an increasing bioavailability of CyA.

Table 2 Overview of 94 6-h pharmacokinetic profiles (mean ± SD and range)

Time after Tx	No. of profiles	C_{max} (ng/ml)	AUC/6 (ng/ml)	TL (ng/ml)
1 (years)	23	599 ± 279	343 ± 124	104 ± 32 (61–175)
2 (years)	21	456 ± 297	255 ± 135	95 ± 36 (37–212)
3 (years)	23	402 ± 168	227 ± 87	89 ± 53 (25–239)
4 (years)	11	412 ± 204	228 ± 91	95 ± 47 (26–178)
≥ 5 (years)	16	451 ± 296	242 ± 148	87 ± 56 (29–261)
Mean		472 ± 262	264 ± 126	95 ± 44 (25–261)

Table 3 AUC/6 (average concontration at steady state) versus CyA toxicity on renal biopsy (mean ± SD)

Biopsy CyA toxicity	N	AUC/6 (ng/ml)	TL (ng/ml)	Serum creatinine (mg/dl)
Absent	11	219 ± 99	81 ± 37	1.31 ± 0.25
Present	3	479 ± 67	149 ± 60	1.46 ± 0.42
Mann-Whitney U-test		$P < 0.05$	NS	NS

It has been shown that children less than 5 years of age have an enhanced immune response to organ transplantation and thus require higher levels of immunosuppression [3]. We found that children weighing less than 25 kg require significantly higher doses of CyA per body weight or body surface to achieve comparable TL than larger children. Previous investigators found higher CyA clearances in children than in adults [4, 13, 19]. The bioavailability of CyA in pediatric and adult recipients appears to be similar [19]. These observations explain why pediatric recipients require substantially higher doses of CyA (mg/kg per day) than adults to achieve comparable serum levels. In fact, failure to achieve therapeutic and effective CyA blood concentrations because of the higher clearances is probably one of the causes of increased graft loss in pediatric renal transplant recipients as compared to adult renal transplant patients [13, 14].

Hoyer et al. [9, 10] reported that a dosage regimen based on body surface area has advantages over a body weight-related dosage regimen. In the present study, our findings could not support this contention. We found a linear correlation between a body surface and body weight-related dosage in the relevant dosage range. Moreover, in our study, the dosage calculated per body surface was also significantly higher for the smaller children than for the larger children during the entire follow-up period.

The variety of profile patterns described in adult patients following renal transplantation was also found in our population: the CyA profile measurements in our children mirrored the adult experience, showing early, middle and late peaks [16, 20].

Since blood concentration time profiles collected over 12 or 24 h providing data on T_{max}, C_{max}, AUC, and average concentration at steady state are difficult to perform in an ambulatory setting, we used abbreviated AUC studies, as introduced by Lindholm and Kahan [16]. The time points were chosen to include TL and to require the patient to be available for only 6 h per day, a sampling schedule more easily applicable in outpatient care. By comparing 29 AUC/12 derived from the 12-h profiles with the AUC/6 derived from the corresponding 6-h profiles, we found a highly significant linear correlation ($r = 0.967$; $P < 0.001$).

Oral absorption of CyA is known to be incomplete and erratic in both adult and pediatric patients [24]. The absorption of CyA in the majority of children is fast. In 75 % of the profiles, maximum concentration (C_{max}) was reached within 2 h after drug intake, while according to Sommer et al. [22], in adults the average time of the first plasma peak observed was 5.7 ± 3.5 h.

In children especially, pharmacokinetic profiles are necessary to discover absorption problems or altered CyA clearances that require dosing regimen adjustments [11, 17, 20]. Since CyA nephrotoxicity can occur after several years of treatment with CyA TL within or even below therapeutic range, it was interesting to observe that patients with nephrotoxicity on biopsy showed the average steady state concentration (AUC/6), which was significantly higher, while neither TL nor serum creatinine differed significantly. Therefore, with regard to the long-term use of CyA, we propose measuring AUC/6 regularly and keeping it below 400–450 ng/ml.

To sum up then, long-term investigation in pediatric transplant recipients illustrates the persistence of wide inter- and intraindividual variability of CyA pharmacokinetic profiles. The abbreviated AUC/6 performed once a year on a clinical, outpatient basis provides more information than TL monitoring alone on appropriate long-term dosage of CyA, especially as related to the rapid elimination in children. Prevention of CyA-associated histological nephrotoxicity in children can best be controlled by a therapeutic monitoring of trough levels associated with regular 6-h AUC monitoring.

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