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Problems of Cyclosporine Absorption Profiling Using C2-monitoring

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Abstract
The present study sought to validate the concept of C2 monitoring in 41 de-novo transplant patients treated with microemulsion of cyclosporine, mycophenolatesodium, steroids and basiliximab.
Results: After 6 months patient and graft survival was 98%, rejection rate was 19%. In the first week only a few patients achieved the suggested C2 levels (19% >1500, 50% > 1200 ng/ml) despite an increased cyclosporine (CsA) dose. After 14 days 63% of patients reached C2 > 1500 ng/ml (83% C2 >1200) despite decreased CsA dose. 35% of patients had intermittent high C0 (>300) and low C2 (<800), suggesting poor and/or slow absorption. Most of them suffered from CsA toxicity. There was a significant ($p<0.05$) change of absorption as measured by C2/C0 leading to an increase of C2/dose.
Conclusions: C2 monitoring may be useful to better estimate the CsA exposure in individual patients; however our results indicate some limitations of the current concept of C2 monitoring. Despite increase of dosage many patients do not reach the proposed levels. A significant proportion of patients are poor and/or slow absorbers. CsA toxicity may not be detected by C2 monitoring alone. With the use of basiliximab and mycophenolatesodium lower target levels seem to be sufficient.

Key words: Cyclosporine, C2-monitoring

Introduction

10 years ago, the highly variable pharmacokinetics of cyclosporine (CsA) were improved by the introduction of the microemulsion of cyclosporine [1]. The more reliable pharmacokinetics of the new formulation allowed the search for better monitoring strategies, because the conventional monitoring by trough level (C0) measurement was not optimal due to a poor correlation with drug exposure or clinical outcome [2].

The sampling of CsA concentration 2 hours post-dose (C2) is supposed to be a better predictor of CsA drug exposure in the individual [3, 4]. Higher C2 levels are correlated with a lower risk of acute rejection in the early posttransplant peroid in patients receiving the cyclosporine emulsion [5]. The present study sought to validate this concept in 41 de-novo renal transplant patients.

Materials and Methods

In an international multicenter trial (Protocol No. CERL080A2405-DE01) a total of 41 de novo renal transplant patients were treated with enteric coated mycophenolic acid (720 mg b.i.d.) in combination with basiliximab (day 0 and 4 post-transplant), steroids and cyclosporine microemulsion, adjusted by C-2h monitoring. Target C2 level were 1500 ng/ml for the first four weeks. Thereafter, patients were randomized into two groups. In the first group the usual cyclosporine microemulsion (CsA) regimen was applied, whereas patients from the second group received a reduced dose of cyclosporine microemulsion. All patients from our centre were included in the analysis of a period of six month post transplantation. However, for the analysis of the relationship between cyclosporine exposure and rejection rates / toxicity, only those drug levels and events were considered, which occurred in the first month after transplantation, a period, when all patients were treated the same way.

Only those CsA levels that were measured in a steady state (at least 36h after dose change) were included in the analysis. C2 sample collection was tolerated 2h $\pm$ 15 min after dosing. CsA concentrations were measured by a specific monoclonal antibody according to the manufacturer´s guidelines (CEDIA assay, Microgenics Corporation Fremont, CA, USA). At regular visits, serum creatinine, concomitant medication and all clinically relevant events such as rejection or CsA toxicity were prospectively documented. A suspected acute rejection was confirmed by biopsy unless clinically contraindicated. CsA nephrotoxicity was defined as an improvement of renal function after CsA dose reduction due to suspected or biopsy proven CsA toxicity. Elevated liver enzymes (GOT/AST 3 x above the normal range) in the presence of elevated CsA levels and improvement of liver function after reduction of CsA dose we defined as CsA liver toxicity.

Mean and standard deviation (SD) of C0 and C2 concentrations and CsA absorptions, expressed as C2/dose, were calculated for all patients over the first 4 weeks. Due to randomisation of patients after four

weeks in groups with different target levels, we did not analyse CsA concentrations after the first month. For all statistical analysis we used the Statistical Program of Social Sciences (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL). All continuous data were expressed as mean ± SD. Differences between group mean values were considered to be significant for a p-value of < 0.05.

Results

After 6 months patient and graft survival was 100% in our cohort of 41 de novo transplanted patients. Acute rejection was suspected in 8 patients (19.5%) and in 6 cases (14.6%) proven by biopsy. Median time to rejection was 11 days after transplantation. Three patients were switched from CsA to Tacrolimus due to severe rejection within the first week posttransplant. Ten (26%) of 38 patients that remained on therapy with CsA showed signs of CsA nephrotoxicitiy. In 5 cases the nephrotoxicitiy was confirmed by biopsy. Liver toxicity, as evidenced by increasing transaminases in the context of high CsA levels, was observed in 19 of 38 patients (50%).

C2 concentrations increased significantly ($p<0.05$) over the first postoperative month (Fig.1). Despite dose changes targeted C2 levels were not reached until week 2. In the first week only 19% of patients achieved C2 target levels of 1500 ng/ml, and 50% of patients reached C2 levels of >1200 ng/ml, despite increasing CsA dose from 623 ± 175 to 672 ± 240 mg/d (Table 1). After two weeks 63% of patients had reached C2 levels of 1500 ng/ml and the most patients (83%) achieved C2 levels of >1200 ng/ml despite decreasing CsA dose to 483 ± 160 mg/d ($p<0.05$). CsA trough levels over the first month were between 250-300 ng/ml. Surprisingly, 11/38 (29%) of patients had intermittend high C0 concentrations (> 300 ng/ml), but at the same time low C2 levels (< 800 ng/ml), suggesting poor and/or slow absorption during the first month posttransplant. Rejection rate in these 11 patients was 18%, compared to a rejection rate of 7.4% in the other patients during the same time period ($p>0.05$). CsA nephro- and/or liver toxicitiy was observed in 7/11 (64%) of those patients with poor absorption, compared to 12/27 (44%) in the other patients ($p>0.05$). C0 concentrations in the patient groups with rejection, and CsA toxicity (liver or nephrotoxicitiy) were not significantly different compared to the other patients, whereas C2 levels and absorption at the time of the event were lower in all groups (Table 1).

Discussion

In the present study we observed an excellent clinical outcome with a graft and patient survival of 100 % six months after transplantation and a rejection rate of 19% over this period. Despite dose adjustments most of the patients did not reach the proposed C2 target levels, particulary in the first two weeks posttransplant, probably due to poor absoption in the initial postoper-

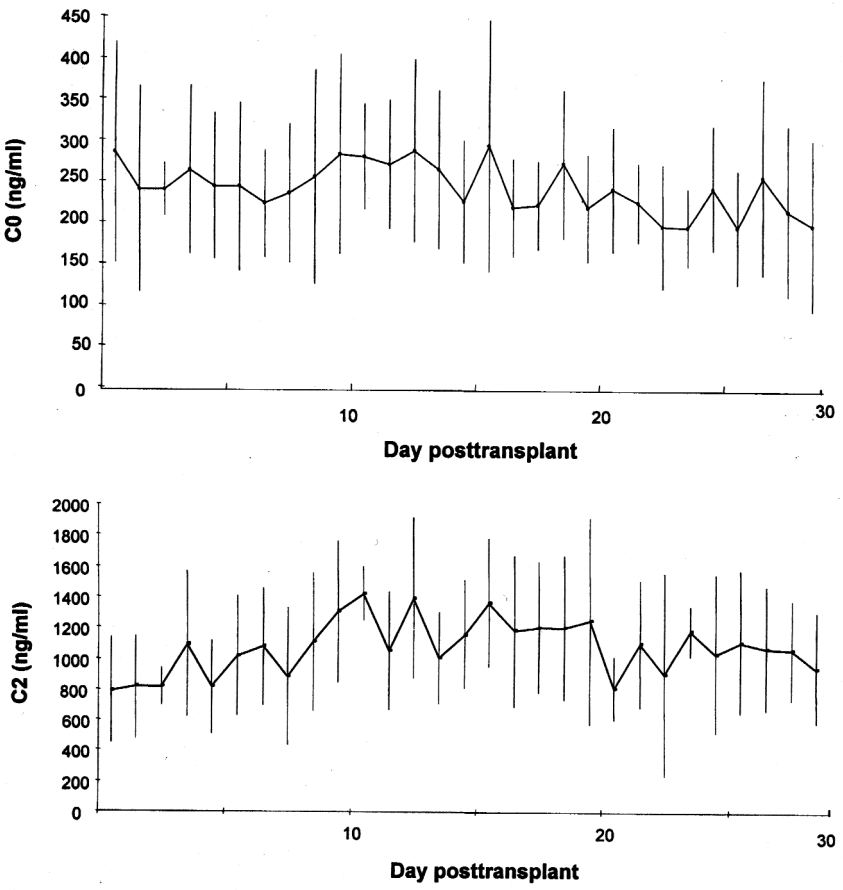

Fig. 1. (a) Mean (± SD) C0 concentrations in 41 patients in the posttransplant period reach quickly adequate levels and are stable over time. (b) Mean (± SD) C2 concentrations are low in the initial postoperative period and increase over time but variability remains high.

Table 1. Mean (SD) of C0, C2 and C2/dose concentrations in different patient groups with rejection, CsA nephrotoxicity or liver toxicity.

	Rejection		Significance
	No	**Yes**	
Mean C0 d 1-30 (ng/ml)	246 ± 49	211 ± 95	n.s.
Mean C2 d 1-30 (ng/ml)	1098 ± 50	705 ± 103	p<0.05
Mean C2/dose d 1-30(ng/ml)(mg/kg)	171 ± 46	128 ± 77	n.s.
	CsA nephrotoxicity		
	No	**Yes**	
Mean C0 d 1-30 (ng/ml)	255 ± 50	237 ± 47	n.s.
Mean C2 d 1-30 (ng/ml)	1095±329	1027 ± 322	n.s.
Mean C2/dose d 1-30 (ng/ml)(mg/kg)	168 ± 50	121 ± 89	p<0.05
	Liver toxicity		
	No	**Yes**	
Mean C0 d 1-30 (ng/ml)	251 ± 57	224 ± 40	n.s.
Mean C2 d 1-30 (ng/ml)	1120 ± 325	936 ± 370	n.s.
Mean C2/dose d 1-30 (ng/ml)(mg/kg)	164 ± 55	111 ± 33	p<0.05

ative period. Our results are consistent with findings of other investigations of CsA absorption [6] which showed that despite high initial CsA doses it is difficult to reach the proposed C2 concentrations. CsA absorption improved significantly over the first month after transplantation. In parallel. we observed an increase of C2 levels between day 8 and 30 despite a decreased CsA dose. These findings may be explained by a time-dependent increase in absolute bioavailability of CsA [7]. After transplant surgery absorption of orally delivered drugs is generally reduced, depending on the type and duration of anesthesia, hydration, reduced bileflow and intestinal motility. Further on a change the CsA absorption can be explained by differences in content and activity of CYP450-3A4 and P-glycoprotein over time [8, 9].

This is the first study to show that a significant proportion of patients (29%) are poor and/or slow absorbers, characterized of low C2 levels (<800 ng/ml) and high C0 concentrations (>300 ng/ml). Despite of the low C2 levels in this subgroup of patients there was not a higher rejection rate compared to the other patients. In contrast, in 64% of these patients we observed signs of CsA toxicity, indicating CsA over-exposure that can not be detected by C2 monitoring alone. To avoid a dangerous over-exposure in the individual patient, we propose the determination of C2 levels should be accompanied by the measurement of CsA trough levels. In addition, our results suggest that low C2 levels do not in all cases represent an increased risk of rejection.

Contrary to previously reported trials [10] we could not confirm an association between C2 levels and clinical event rate in our patient cohort. Most probably the effective immunosuppressive co-medication with basiliximab and mycophenolate sodium prevented the occurence of acute rejection in the early postoperative perid despite low CsA exposure.

In summary, C2 monitoring may help to better estimate the CsA exposure in individual patients; however our results indicate some limitations of the current concept of C2 monitoring. There is high variability in CsA absorption as detected by C2 monitoring, with an increase of CsA absorption in the initial postoperative period. Despite increase of dosage many patients do not reach the proposed levels. A significant proportion of patients are poor and/or slow absorbers. CsA toxicity may not be detected by C2 monitoring alone. Further investigations are warranted before its widespread adoption. Under effective immunosuppression with basiliximab, mycophenolate sodium and steroids, lower CsA exposure and lower C2 levels are sufficuient for excellent clinical outcomes.

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