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Increase in tacrolimus trough levels after steroid withdrawal

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Abstract Although there are experimental reports of cytochrome P450 3A4 iso-enzyme (CYP3A4) induction by glucocorticoids, there are no clinical reports about an interaction between tacrolimus and steroids. Therefore, tacrolimus trough level and dose were compared after dose-normalization before and after withdrawal of prednisolone. After withdrawal of 5 mg prednisolone, the median tacrolimus dose-normalized level increased by 14% in the retrospective ($n=54$), and by 11% in the prospective ($n=8$) part of the study. After withdrawal of 10 mg, this increase was 33% ($n=30$) and 36% ($n=14$), respectively. An additional pharmacokinetic part of the study ($n=8$) revealed an 18% increase in AUC ($P=0.05$) after withdrawal of 5 mg prednisolone, which is compatible with a reduced metabolism after steroid withdrawal. The significant increase in tacrolimus exposure after steroid withdrawal may on the one hand counteract the reduction in immunosuppression intended by steroid withdrawal, and, on the other hand, may result in an increase of serum creatinine which could be misinterpreted as rejection.

Keywords Tacrolimus levels · Steroid withdrawal · Renal transplantation · Pharmacokinetics

Introduction

Tacrolimus effectively prevents acute rejection after renal transplantation [6, 8, 13, 14]. Generally, it is combined with steroids, both initially and during maintenance therapy. The Pittsburgh group and other investigators [3, 5, 11, 16, 19] have shown that steroids can be withdrawn after renal transplantation in a large proportion of patients. In our center, too, steroids were withdrawn without provoking rejection, in a majority of patients who had been treated with tacrolimus and steroids for more than 3–6 months, had stable graft function and no proteinuria [2]. In several of these patients, we observed an increase in tacrolimus trough levels following steroid withdrawal, although the tacrolimus dose was unchanged.

An interaction between steroids and tacrolimus has been described in *in vitro* studies and in *in vivo* animal studies [9, 10, 12, 15, 18]. Tacrolimus is metabolized by the cytochrome P450 3A4 (CYP3A4) iso-enzyme. Glucocorticoids are inducers of CYP3A4 and consequently would be expected to increase the metabolism of tacrolimus [17]. P-glycoprotein is responsible for an efflux of tacrolimus from the enterocytes back into the gut lumen. Hitherto, it is unclear whether interaction on the P-glycoprotein (P-gp) level could also play a role [7].

Since increase in systemic exposure to tacrolimus after steroid withdrawal has not yet been described, we studied the effect of withdrawal of two different steroid dosages on the systemic exposure to tacrolimus. The evaluations were made both by retrospective and

prospective examination of tacrolimus trough level and corresponding dosing data, as well as by means of a pharmacokinetic study.

Material and methods

Patients

Renal allograft recipients that had been on tacrolimus-based immunosuppression for at least 3 months posttransplantation were enrolled. Patients with a history of acute rejection (BANFF 2 or higher), steroid-resistant rejection, hyperimmunized state, unstable renal function or proteinuria, were excluded from steroid withdrawal.

In the retrospective part of the study, all patients with steroid withdrawal who met these criteria were evaluated. In 54 Causcasian patients, 5 mg prednisolone per day, and in 30 Caucasian patients, 10 mg prednisolone per day were stopped. Patients with changes in co-medication were excluded, as were patients whose trough levels had been collected less than 11 or more than 13 h after the last tacrolimus ingestion. In the prospective part of the study, 8 Caucasian patients were evaluated before and after withdrawal of 5 mg prednisolone, and 14 Caucasian before and after withdrawal of 10 mg prednisolone. Additionally, in 8 patients pharmacokinetic parameters were prospectively compared before and after withdrawal of 5 mg of prednisolone, while the tacrolimus dose was left unchanged. Changes in co-medication were not allowed. Trough levels were taken between 11.45 and 12.15 h after the last tacrolimus ingestion. All patients gave informed constent, and the studies were approved by the ethical committee of the hospital. Patient characteristics are shown in Table 1.

Retrospective evaluations

Steady-state tacrolimus whole blood 12-h trough levels and the corresponding daily doses were recorded before tapering of steroids and after complete cessation of steroids. Steady state was assumed when the daily dose of tacrolimus was stable (unchanged) for three or more days before the measurement of the trough level. Blood levels taken shortly before the start of steroid reduction, and those taken shortly after complete cessation of steroids that met the steady-state definition were used for this study. To account for changes in tacrolimus dose, comparisons were made between the dose-normalized concentrations (tacrolimus trough level divided by the corresponding daily dose).

Prospective evaluations

In the patients receiving 5 mg prednsisolone, 209 days (151–864) after transplantation, prednisolone was tapered to 2.5 mg/day for 1 week and thereafter completely stopped.

In the patients receiving 10 mg prednisolone, after obtaining normal ACTH stimulation tests, the daily prednisolone dose was reduced from 10 mg to 5 mg for 1 week, to 2.5 mg for the following week, and thereafter competely stopped. Tacrolimus doses and tacrolimus trough levels that met the steady-state definition, and serum creatinine levels were evaluated at the same time at the start of steroid withdrawal and after complete cessation of steroids.

Immediately before, and 1 week after complete cessation of steroids, tacrolimus blood concentration-time profiles were taken over a 12-h dosing period. Blood samples were taken at pre-dose (0) and then at 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 9.0 and 12.0 h post-dose. For a period of 1 week before the first pharmacokinetic profile until after the second profile, the daily dose of tacrolimus had to be unchanged. Tacrolimus was administered after an overnight fast. A standard breakfast containing 1928 kJ of energy, 43% fat content was given after the withdrawal of 1-h blood.

Concentrations of tacrolimus in whole blood were determined by IMx (Abbott, Hoofddorp, the Netherlands). Blood concentration-time data were used to calculate standard pharmacokinetic parameters [Cmax, tmax, oral clearance and AUC 0-12, using the computer program MWPHARM 3.30 (Mediware, Groningen, the Netherlands)].

Statistics

For statistical analysis, SPSS version 10.0 for Windows (Cary, Chicago, Ill.) was used. To compare dose-normalized level and pharmacokinetic parameters before and after steroid withdrawal, Wilcoxon matched-pairs signed rank sum test was performed. Unless indicated otherwise, data are given as median and range. A P-value below 0.05 was considered to be statistically significant.

Results

Tacrolimus dose, trough level, and dose-normalized level before and after steroid withdrawal are presented in Table 2.

In the retrospective part of the study, steroid tapering started at a median of 262 days (range 88–1626) after transplantation, and was completed in 100 days (0–208)

Table 1 Basic patient characteristics. *LRD* Living related donor, *LUD* Living unrelated donor, *Immunosupp* other immunosuppressive agents. Data are given as numbers or median (+range)

	Retrospective part		Prospective part	
Steroid dose (mg)	5 mg	10 mg	5 mg	10 mg
Number of patients	54	30	8	14
Gender: male/female	34/20	23/7	7/1	10/4
Tx number: first/retransplant	44/12	25/5	6/2	13/1
Tx type: LRD/LUD/cadaveric	0/6/48	1/2/27	0/1/7	1/1/12
Immunosupp: AZA/MMF/none	8/4/44	0/13/17	0/1/7	0/14/0
Diabetes mellitus	4	5	0	1
Age (years)	51.5 (15.9–67.5)	57.0 (22.0–72.0)	50.9 (32.7–67.8)	60.5 (32.0–75.0)
Body mass index (kg/m^2)	24.7 (16.6–36.5)	24.5 (15.6–31.3)	25.8 (18.8–34.7)	25.2 (17.1–31.7)
Time after tx (days)	262 (88–1626)	242 (127–393)	209 (151–864)	90 (85–92)

Table 2 Tacrolimus dose and trough level before and after steroid withdrawal. Median and ranges of tacrolimus trough levels, tacrolimus dose and dose-normalized level before and after steroid withdrawal

			Before steroid withdrawal	After steroid withdrawal	$P=$
Retrospective	5 mg prednisolone	trough level (ng/ml)	8.5 (4.3–17.2)	8.3 (3.6–25.9)	
	$n=54$	dose (mg/day)	6.0 (2.0–20.0)	5.0 (2.0–19.0)	
		dose-normalized level (ng/ml per mg/day)	1.4 (0.5–4.3)	1.6 (0.6–7.8)	0.003
	10 mg prednisolone	trough level (ng/ml)	7.8 (4.2–25.6)	9.4 (5.3–16.8)	
	$n=30$	dose (mg/day)	5.0 (2.0–14.0)	4.5 (2.0–14.0)	
		dose-normalized level (ng/ml per mg/day)	1.5 (0.5–6.4)	2.0 (0.6–4.9)	0.011
Prospective	5 mg prednisolone	trough level (ng/ml)	7.3 (3.8–9.9)	8.1 (5.0–14.1)	
	$n=8$	dose (mg/day)	5.0 (2.5–13.0)	5.0 (2.5–13.0)	
		dose-normalized level (ng/ml per mg/day)	1.6 (0.4–4.0)	1.8 (0.6–5.6)	0.058
	10 mg prednisolone	trough level (ng/ml)	8.3 (4.9–14.5)	11.9 (7.6–15.0)	
	$n=14$	dose (mg/day)	5.5 (3.0–15.0)	5.5 (4.0–16.0)	
		dose-normalized level (ng/ml per mg/day)	1.4 (0.2–2.9)	1.9 (0.8–3.5)	0.002

in patients receiving 5 mg prednisolone per day. In patients on 10 mg prednisolone per day, steroid tapering started 242 days (127–393) after transplantation, and was completed in 68 days (6–311). Dose-normalized level increased by 14% after withdrawal of 5 mg prednisolone, from 1.4 to 1.6 ng/ml per mg ($P=0.003$), and by 33% after withdrawal of 10 mg prednisolone, from 1.5 to 2.0 ng/ml per mg ($P=0.011$).

In the prospective part of the study, 8 patients were evaluated 7 days after withdrawal of 5 mg prednisolone, 209 days (151–864) after transplantation, and 14 patients were evaluated before and 17 days (13–31) after withdrawal of 10 mg prednisolone, 90 days (85–92) after transplantation. Dose-normalized levels increased by 12% after withdrawal of 5 mg prednisolone, from 1.6 to 1.8 ng/ml per mg ($P=0.058$), and by 36% after withdrawal of 10 mg prednisolone, from 1.4 to 1.9 ng/ml per mg ($P=0.002$). After withdrawal of 10 mg prednisolone, serum creatinine increased from 143 umol/l (67–246) to 151 umol/l (67–259) ($P=0.034$).

Figures 1 and 2 show the relative changes in tacrolimus dose-normalized levels after withdrawal of 5 and 10 mg prednisolone in all patients. Increases of more than 20% occurred in 43% of the patients after withdrawal of 5 mg, and in 61% of patients after withdrawal of 10 mg prednisolone.

Table 3 shows the pharmacokinetic parameters before and after steroid withdrawal. The median AUC increased from 132.3 ng.h/ml before steroid withdrawal to 156.6 ng.h/ml after steroid withdrawal ($P=0.05$). The corresponding values for oral clearance were 0.280 and

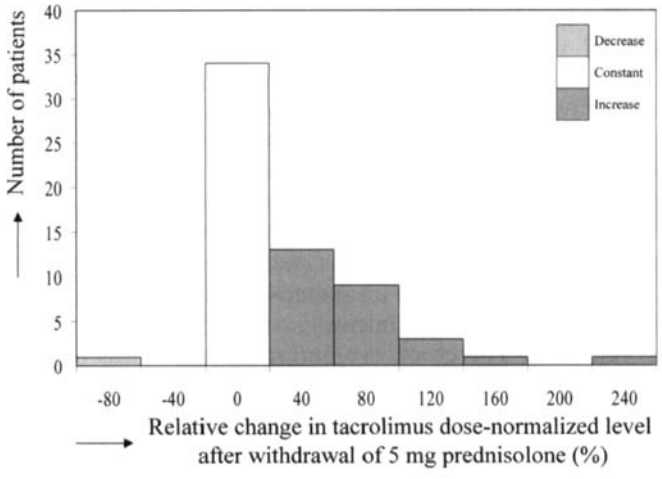

Fig. 1 Relative changes (%) in tacrolimus dose-normalized level after withdrawal of 5 mg prednisolone for all retrospective and prospective patients ($n=62$). Tacrolimus dose-normalized level was defined as tacrolimus trough level divided by corresponding daily dose. A decrease in tacrolimus dose-normalized level was defined as a reduction of tacrolimus dose-normalized level of 20% or more, and an increase as an addition of 20% or more, when tacrolimus dose-normalized level had changed less than 20%, this was regarded as no change

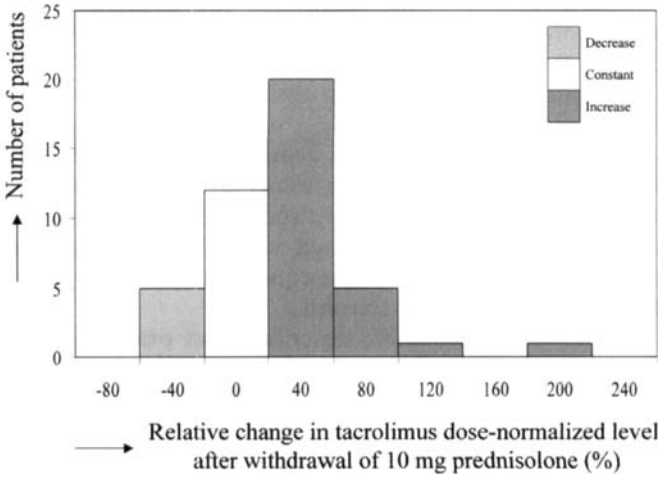

Fig. 2 Relative changes (%) in tacrolimus dose-normalized level after withdrawal of 10 mg prednisolone for all retrospective and prospective patients ($n=44$). The Tacrolimus dose-normalized level was defined as tacrolimus trough level divided by corresponding daily dose. A decrease in tacrolimus dose-normalized level was defined as a reduction of 20% or more, and an increase as an addition of 20% or more. Changes in the dose-normalized tacrolimus level under 20% were regarded as no change

Table 3 Pharmacokinetic parameters before and after steroid withdrawal for each patient. Tacrolimus trough level, C_{max} (the maximum trough level after tacrolimus ingestion), t_{max} (the time after transplantation when the maximum trough level was reached), oral clearance and AUC (area under the curve, total tacrolimus exposure)

	Trough level (ng/ml)		C_{max} (ng/ml)		t_{max} (h)		Oral clearance (l/h/kg)		AUC (ng.h/ml)	
	pre	post	pre	post	pre	post	pre	post	pre	post
Patient 1	8.3	7.9	22.5	26.9	1.00	1.00	0.293	0.238	135.1	166.8
Patient 2	9.2	8.0	28.2	27.3	1.00	1.00	0.291	0.338	170.1	146.4
Patient 3	4.6	5.0	16.0	12.2	1.00	1.00	0.251	0.267	99.1	94.1
Patient 4	6.3	6.9	19.6	23.7	0.45	1.00	0.268	0.178	94.5	140.8
Patient 5	9.0	10.4	30.9	42.3	1.00	1.00	0.206	0.188	185.2	201.9
Patient 6	3.8	8.4	36.9	37.8	1.00	1.00	0.413	0.269	129.6	184.0
Patient 7	9.9	14.1	27.3	29.8	1.00	1.00	0.133	0.099	175.4	237.8
Patient 8	5.4	8.1	15.6	19.2	2.00	2.00	0.619	0.584	103.3	136.6
Median	7.3	8.1	24.9	27.1	1.00	1.00	0.280	0.253	132.3	156.6
P	0.08		0.12		0.32		0.05		0.05	

0.253 l/h per kg ($P=0.05$), trough levels were 7.3 and 8.1 ($P=0.08$) and Cmax were 24.9 and 27.1 ($P=0.12$).

Discussion

Based on our clinical observations and supported by reports about a possible interaction between tacrolimus and steroids [7, 9, 10, 12, 15, 17, 18], we evaluated the effect of steroid withdrawal on the systemic exposure to tacrolimus.

The results of our evaluations have confirmed that the withdrawal of steroids results in an increased systemic exposure to tacrolimus. The increase in systemic exposure to tacrolimus following the withdrawal of 10 mg prednisolone was higher (33–36%) than the increase after withdrawal of 5 mg prednisolone (12–14%). The increase in AUC that is observed is not associated with an increase in either C_{max} or t_{max}, suggesting that the processes of absorption remain unaffected. Therefore, a role of P-glycoprotein seems less likely. The most likely cause for the increased exposure following steroid withdrawal may be associated with a decrease in the metabolic clearance as a consequence of the reversal of CYP3A4 induction by steroids.

In an earlier study we described that oral clearance decreases over time [4]. From the current study we can conclude that, besides increasing hemoglobin and albumin levels, the marked steroid reduction in this period, too, explains the decrease in oral clearance over time. Furthermore, the current study suggests that the effect of steroid withdrawal is steroid dose-dependent. After withdrawal of only 10 mg prednisolone, an increase in the tacrolimus dose-normalized ratio of ± 35% was found. Generally, much higher steroid doses are used in renal transplantation. After withdrawal of such higher steroid doses, an even larger increase in tacrolimus trough levels might be expected.

The assay method used to determine tacrolimus blood (Imx, Abbott) is reported to have a precision of up to ± 20% at 5 ng/ml [20, 21]. Therefore, an increase in blood level of > 20% was considered to be relevant. Such an increase occurred in 43% of the patients after withdrawal of 5 mg prednisolone, and 61% after withdrawal of 10 mg prednisolone. In individual patients, the increase amounted to as much as 200%. This indicates that the increase in tacrolimus trough level after steroid withdrawal is a clinically important issue.

The increase in systemic exposure to tacrolimus after steroid withdrawal may on one hand counteract the reduction in immunosuppression intended by steroid withdrawal, and, on the other hand, will result in an increase of serum creatinine in some patients. This was observed in an earlier study [1], where we found a significant increase in creatinine clearance by 16% after a 33% reduction of tacrolimus levels, from 9.5 to 6.4 ng/ml, and was confirmed in this study. Such an increase of creatinine levels due to an increase in tacrolimus trough levels after steroid withdrawal could be misinterpreted as rejection. Therefore, evaluation of tacrolimus trough levels within 1–2 weeks after steroid withdrawal is advisable for all patients. Tacrolimus dose should be reduced in all patients with a significant increase in trough level after steroid withdrawal. This is obviously also financially beneficial.

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