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ORIGINAL ARTICLE

Evolution of tacrolimus blood levels and concentration-dose ratios in patients who develop new onset diabetes mellitus after kidney transplantation

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Summary

New onset diabetes mellitus (NODM) affects kidney transplantation outcome. Several risk factors, including immunosuppressive drug levels, are related with NODM development. This analysis evaluates the incidence and risk factors of NODM in kidney transplant patients receiving tacrolimus, taking into account 6-month blood levels and concentration-dose ratios (CDRs). Seventy-six patients under tacrolimus therapy who received a cadaveric renal transplant in our centre and with graft survival higher than 1 year were included in the study. NODM was defined as two fasting plasma glucose values ≥126 mg/dl or symptoms of diabetes plus casual plasma glucose concentrations ≥200 mg/dl throughout the first year. We examined previously reported variables related with NODM development. The incidence of NODM at 12 months was 27.6%. Risk factors for NODM included older age, higher first tacrolimus level, higher body mass index and lower first year weight gain. In multivariate analysis, the first year occurrence of NODM was significantly determined by the first tacrolimus blood level >20 ng/ml and age older than 50 years. CDR remains significantly higher in NODM throughout the 6 months. Older age and a high first tacrolimus blood level are associated with the development of NODM during the first year after kidney transplantation. NODM patients show higher CDR during the first 6 months.

Introduction

New onset diabetes mellitus (NODM) has a high incidence after kidney transplantation and may adversely affect the patient and graft survival [1,2]. Factors associated with increased risk for developing NODM are ethnicity, family history of diabetes, obesity, glucose intolerance, hepatitis C virus infection, older age, cadaveric donor and immunosuppressive drugs [2]. Tacrolimus is a macrolide with potent immunosuppressive effects and highly effective for prevention of acute rejection (AR), but is particularly diabetogenic when used as an initial immunosuppressant [3]. Initial blood concentrations are important for reducing the AR rate and secondary effects such as NODM [4,5]. The purpose of our study was to know the incidence of NODM in the first year of kidney transplant patients under tacrolimus therapy and the risk factors for NODM, taking into account the doses and concentrations of tacrolimus and their evolution during the first few months.

Patients and methods

Seventy-six patients treated with a tacrolimus-based immunosuppressive therapy who received a cadaveric renal transplant in our centre between January 1994 and

February 2003 and with graft survival higher than 1 year were included in the study. During this period of study, 578 kidney transplants were performed in our centre. Approximately 128 patients received tacrolimus from the first day, but only 101 survived graft longer than 1 year or else received tacrolimus throughout the first year. Of these, 10 patients were excluded because of pretransplant diabetes mellitus, six due to previous glucose intolerance during renal replacement therapy and nine had no adequate data (absence of registered tacrolimus levels, weight or height). Immunosuppressive induction therapy consisted of corticosteroids, tacrolimus and mycophenolate mophetil (MMF) or azathioprine (AZA). Daily initial tacrolimus dosage was usually 0.2 mg/kg, divided into two doses. Patients receiving a kidney from an older donor or developing DGF after transplantation usually received half-tacrolimus doses. Tacrolimus doses were adjusted to achieve blood target levels of 10–15 ng/ml during the initial 3 months after transplantation. MMF was started at a dose of 1 g/day and AZA at a dose of 1.5–2 mg/kg/day.

The presence of NODM was defined as two fasting plasma glucose values ≥126 mg/dl or symptoms of diabetes plus casual plasma glucose concentrations ≥200 mg/dl throughout the first year [6]. Elevated blood glucose levels early after transplantation caused by high-dose steroid therapy and intravenous glucose infusions during the first week or during AR therapy were not considered to define a patient as diabetic. The necessity of drug therapy for NODM (DT-NODM) with insulin or oral antidiabetics was also collected.

We examined a number of variables to determine whether they were associated with NODM development. These variables included: recipient's age and sex, donor's sex, body mass index (BMI), first year weight gain, human leucocyte antigen mismatches, type of dialysis, hepatitis C antibody status at transplantation, delayed graft function (DGF) and AR episodes, annual cumulative dose of corticosteroids, steroid withdrawal during the first year, use of MMF or AZA and statin use. All patients included were Caucasians.

Tacrolimus trough concentrations were measured by microparticle enzyme immunoassay, always obtained after at least five stable doses of tacrolimus. Concentration-dose ratios (CDRs) were calculated dividing tacrolimus blood level (ng/ml) by the previous daily tacrolimus dose (mg/day) and their units were (ng/ml)/(mg/day). Tacrolimus blood levels, doses and CDRs were collected according to the following scheme: first tacrolimus level after transplantation, first level in the therapeutic range (under 15 ng/ml), and first monthly level from months 2 to 6 after transplantation.

Statistical analyses were carried out using SPSS 8.0 software. Mean values in the two groups (NODM and non-NODM) were compared by the Student's *t*-test or by nonparametric tests (Mann–Whitney) if such data were not normally distributed. Proportions were compared by chi-square analysis. We examined the independent relationship between variables and NODM using Cox's proportional hazards analysis. Results were considered statistically significant for $P < 0.05$.

Results

The incidence of NODM at 12 months was 27.6% (21 patients, 95% CI 18.0–39.1%), while the incidence of DT-NODM was 14.4% (11 patients, 95% CI 7.5–24.4%). Out of DT-NODM patients, nine were under insulin therapy and only two received oral hypoglycaemic agents. Five patients developed NODM in the first 2 months, but presented spontaneous regression during the follow-up and they were considered as one-year non-NODM for analysis.

Risk factors for NODM included older age, higher first tacrolimus level, higher BMI and lower first year weight gain. There were no statistically significant differences in tacrolimus doses, tacrolimus weight-adjusted doses, first tacrolimus blood levels in the therapeutic range, tacrolimus levels from months 2 to 6, donor and recipient sex, dialysis method, incidence of DGF and AR, use of MMF or AZA, steroid withdrawal rate, annual cumulative dose of corticosteroids and statin use. The presence of hepatitis C antibody was higher in patients with NODM, but without statistical significance (Table 1). Figures 1 and 2 show the evolution of tacrolimus blood levels and CDRs for the first 6 months in NODM and non-NODM patients. Only the first level of tacrolimus was significantly higher in NODM patients, while all the other blood level values showed no differences between NODM and non-NODM. By contrast, CDR remains significantly higher in NODM.

In multivariate analysis, NODM development in the first year after transplantation was significantly determined by the first tacrolimus blood level >20 ng/ml and age over 50 years. Neither BMI nor first year weight gain proved significant after regression (Table 2).

Discussion

NODM incidence at 12 months after kidney transplantation varies from 2% to 50% according to different studies [7]. In a meta-analysis of randomized trials, Knoll and Bell [8] reported a prevalence of one-year NODM from 2.0% to 12.3% . Incidence varies depending on the date of transplantation, the studied population, the diabetes definition employed and other variables such as

Table 1. Variables of the NODM and non-NODM patients.

	NODM ($n = 21$)	Non-NODM ($n = 55$)	P-value
Age (years)	58.1 ± 9.8*	43.9 ± 13.7	<0.001
Age >50 years %	85.7*	32.7	<0.001
Recipient sex (male) %	57	70	0.253
Donor sex (male) %	58	66	0.594
BMI (kg/m^2)	27.6 ± 4.0*	24.7 ± 4.0	0.006
BMI >25 kg/m^2 %	80.9*	50.9	0.017
First year weight gain (kg)	−3.7 ± 8.1*	4.2 ± 6.9	<0.001
MMF %	84	80	0.313
Statins %	23.8	29.0	0.645
Mismatches	3.6 ± 1.0	3.6 ± 1.0	0.842
Delayed graft function %	33	25	0.752
Acute rejection %	14.2	20	0.566
Hepatitis C virus %	14.2	3.6	0.094
Dialysis method (haemodialysis) %	80	76	0.757
Steroid withdrawal %	19.0	7.2	0.135
First year cumulative steroid dose (g)	4.0 ± 1.3	4.3 ± 1.6	0.483
First tacrolimus blood level (ng/ml)	24.0 ± 7.2*	16.2 ± 6.0	<0.001
First tacrolimus level >20 ng/ml	80.9%*	21.8%	<0.001
First CDR (ng/ml)/(mg/day)	2.0 ± 1.0*	1.3 ± 0.5	<0.001
Initial daily dose (mg)	12.4 ± 3.1	12.9 ± 3.7	0.628
Weight-adjusted initial daily dose (mg/kg)	0.17 ± 0.04	0.18 ± 0.03	0.323
Tacrolimus blood level under 15 ng/ml (ng/ml)	12.6 ± 1.9	11.9 ± 2.2	0.181
Month 2 tacrolimus blood level (ng/ml)	11.8 ± 2.8	10.6 ± 3.1	0.181
Month 3 tacrolimus blood level (ng/ml)	11.3 ± 3.1	10.6 ± 3.3	0.449
Month 4 tacrolimus blood level (ng/ml)	11.8 ± 3.3	10.6 ± 4.3	0.369
Month 5 tacrolimus blood level (ng/ml)	10.6 ± 3.7	10.3 ± 2.1	0.767
Month 6 tacrolimus blood level (ng/ml)	10.0 ± 4.8	9.9 ± 2.9	0.881
CDR with tacrolimus blood level under 15 ng/ml (ng/ml)/(mg/day)	1.9 ± 1.1*	1.2 ± 0.6	0.001
Month 2 CDR (ng/ml)/(mg/day)	2.3 ± 1.0*	1.2 ± 0.6	<0.001
Month 3 CDR (ng/ml)/(mg/day)	2.2 ± 0.9*	1.3 ± 0.6	<0.001
Month 4 CDR (ng/ml)/(mg/day)	2.9 ± 1.3*	1.3 ± 0.7	<0.001
Month 5 CDR (ng/ml)/(mg/day)	2.6 ± 1.0*	1.3 ± 0.5	<0.001
Month 6 CDR (ng/ml)/(mg/day)	2.6 ± 1.3*	1.3 ± 0.5	<0.001

*P-value significant under 0.05.

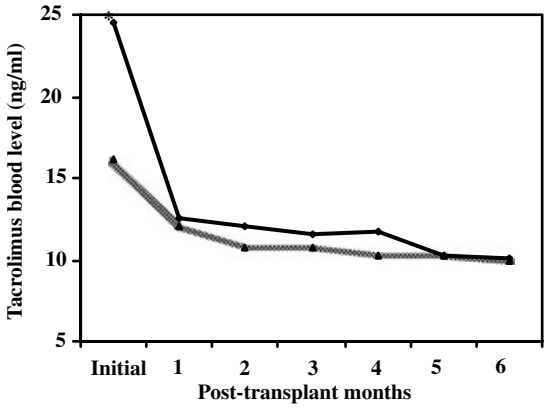

Figure 1 Six-month blood levels of tacrolimus in new onset diabetes mellitus (NODM) (black line) and in non-NODM (grey line) patients. *$P < 0.05$.

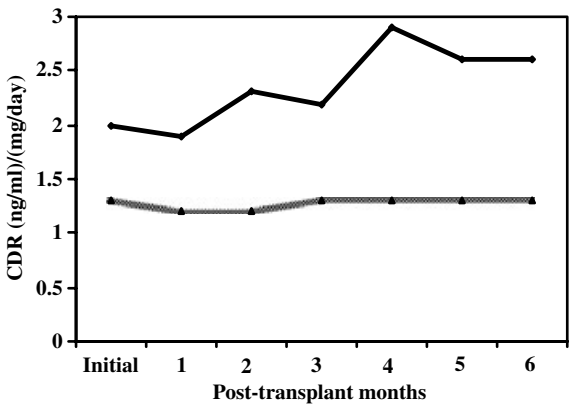

Figure 2 Six-month concentration-dose ratio in NODM (black line) and non-NODM (grey line) patients. Differences remain significant throughout the 6 months.

Table 2. Independent risk factors of NODM defined by Cox's regression model.

	Relative risk	95% Confidence interval	*P*-value
Age >50 years	5.852	1.063–32.224	0.042
First tacrolimus level >20 ng/ml	9.331	2.289–38.031	0.001
BMI >25 kg/m^2	1.060	0.190–5.892	0.946
First year weight gain	0.901	0.802–1.008	0.071

immunosuppressive drugs and their dosage. For instance, Cosio *et al.* [9] reported in a single centre study that recently transplanted patients suffer an increase in the incidence of NODM after 1995, only partly explained by changes in the characteristics of the recipients, such as older age and greater weight . In contrast, Montori *et al.* [7] reported that the incidence of NODM was lower than it had been 3 decades before, after reviewing 19 studies. NODM developed in only 3.2% in a southern European population of tacrolimus-treated patients defining DM as insulin requirement >30 days, whereas 57.1% of Korean transplant recipients were diagnosed as diabetic after a 75-g-oral-glucose tolerance test [10,11]. Using the new criteria for diagnosis of DM, based on fasting plasma glycaemia, we found an incidence of NODM as high as 27.6% at 12 months in our patients receiving tacrolimus, while the rate of transplant recipients who required insulin or oral antidiabetics was 14.4%. These findings are similar to those reported by Maes *et al.* [4] (NODM 32%, DT-NODM 12%) in 139 European patients under tacrolimus therapy using the same diabetes criteria .

Several risk factors have been considered as contributing to the development of NODM: age, BMI, ethnic origin, diabetic family history, cadaveric donor, sex of the donor, number of mismatches, AR episodes, steroid dose, hepatitis C positive patients, impaired glucose tolerance before transplantation, cyclosporine and tacrolimus use and doses, even nonstatin use [1,3,7,9,12–14]. Unfortunately, because of the retrospective nature of our study, it was impossible for us to know diabetic family history. In a univariate analysis, we found that patients who developed NODM 12 months after transplantation were older and heavier at transplant and their initial blood tacrolimus levels were higher. Besides, NODM patients lost weight during the first year, while non-NODM patients gained weight. No other variables studied were significant.

After multivariate analysis, age remains an important risk factor for NODM. Patients over 50 are five times more likely to develop NODM. Age is the only risk factor unanimously reported in all studies, although it is a non-modifiable factor [1,3,7,9,12,13]. We found no differences in the number of mismatches, sex of the donor, statin use and hepatitis C status as previously reported in large studies [1,12,13], probably because of the size of the sample. Hepatitis C virus positivity was more frequent in NODM (14.2%) than in non-NODM (3.6%), but without statistical significance. Neither did we find any difference in the cumulative steroid doses, rate of steroid withdrawal and AR episodes between NODM and non-NODM patients, although the association between corticosteroid therapy and the development of diabetes is clearly established [2]. After the introduction of cyclosporine an association between glucocorticoid pulse therapy (more than the cumulative dose), as treatment for AR and NODM has been reported [7,13].

Weight and BMI at transplant are recognized risk factors for NODM [1,3,4,9,12], even if these are not significant in all reviews [7,13]. We found that overweight, not only obese, patients, were at risk of NODM, although this finding did not remain significant after regression. Weight is the most potentially modifiable risk factor for NODM in patients on the waiting list for transplantation. Lifestyle intervention reduces the risk of type 2 diabetes in populations with elevated glucose levels [15]. In our unit, physicians and nurses encourage high-risk patients (obese, NODM) to lose weight with appropriate diet and increasing physical activity. We have found that patients who develop diabetes lose, rather than gain, weight in the first year. Although it can be explained by medical intervention directed to limit cardiovascular risk factors, it is more probable that the small sample size influences this finding. It is important to highlight that weight loss on the waiting list is most useful to reduce the risk of NODM than after transplantation. Likewise, in a larger study Gourishankar *et al.* [13] have reported no significant difference in weight changes between the recipients who developed NODM as compared with those who did not.

Tacrolimus was used in high doses in phase II and III studies, in which a significantly higher incidence of NODM was revealed. Increasing whole-blood tacrolimus trough concentrations were related with the development of NODM [7,16–18]. In fact, tacrolimus doses have been reduced over the past decade in an attempt to optimize the risk/benefit ratio. Linear regression data reported by Heisel *et al.* [19] show an apparent trend towards a reduction in average NODM rate, although it is difficult to establish the statistical precision of this trend.

We have observed an association between higher initial blood levels and the subsequent development of diabetes while further levels are not so related. Maes *et al.* [4] reported that the number of levels >15 ng/ml during the first month was a significant risk factor for NODM and impaired fasting glycaemia in the first year. In a similar way, we have found that only the first level of tacrolimus favours NODM occurrence, and levels after that have no influence. Usually, transplant teams are successful in

maintaining tacrolimus levels in the nontoxic target range after the first doses. In a recent paper, Gourishankar *et al.* [13] reported no association among 1-month, 6-month and 1-year tacrolimus trough levels and the development of diabetes.

More precisely, transplant recipients with higher first tacrolimus levels are usually older and overweight [20]. The effects of risk factors for NODM are additive. If further prospective studies confirm the diabetogenic toxicity of the first tacrolimus level, overweight and older patients could benefit more from less diabetogenic immunosuppression such as based on cyclosporine, or must receive lower initial tacrolimus doses based on ideal rather than real weight in order to reduce NODM-incidence. Specific studies must be undertaken to define the correct doses and to analyse the impact of this measure in reducing NODM-incidence.

From the first level to month 6 after transplantation we have found that CDR is significantly higher in patients who will develop NODM. A similar finding was suggested by Maes *et al.* [4]. CDR is influenced in tacrolimus-treated patients by several factors. Corticosteroids induce both cytochrome P450 (CYP3A) and P-glycoprotein. The higher the steroid dosage, the higher the dosage of tacrolimus needed to achieve target trough levels and lower CDRs [21]. In our study, NODM and non-NODM patients received similar steroid dosages, and the differences cannot be explained. Multidrug resistance-1 gene polymorphisms and cytochrome P450 3A5 polymorphisms predict CDR and the initial daily dose needed to obtain adequate levels [22,23], but there is no study relating these polymorphisms with diabetes mellitus and we are unaware of their distribution in our group of patients.

As CDR is higher in patients who will develop NODM, a possible explanation is that these patients show higher areas under curve for the same trough concentration, allowing tacrolimus to exert more potent diabetogenic effects. On the contrary, patients prone to diabetes could have an altered absorption or metabolism favouring higher CDR. In this sense, it is known that type-2 diabetes mellitus patients have a lower activity of CYP3A4 that alters the disposition of nisoldipine enantiomers and could increase CDR [24]. Hence, CDR could be more a 'marker' than a risk factor for developing NODM. Studies should be undertaken to know absorption, metabolism and pharmacokinetics of patients prone to NODM.

As a result of the impact of NODM in graft survival and morbidity after kidney transplantation, it is necessary to know exactly the risk factors to reduce its incidence. If further prospective studies confirm the diabetogenic toxicity of the first tacrolimus level, overweight and older patients could benefit more from less diabetogenic immunosuppression such as based on cyclosporine, or must receive lower initial tacrolimus doses based on ideal rather than real weight. Studies must be conducted to determine the most adequate initial doses in this group of patients and the reason for the higher CDR in patients who will develop NODM.

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