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ORIGINAL ARTICLE

The effect of different immunosuppressive regimens on TGF-β1 expression in kidney transplant patients

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Summary

Transforming growth factor (TGF)-β1 is a key profibrogenic cytokine associated with the pathogenesis of chronic allograft nephropathy (CAN). The primary aim of this study was to evaluate TGF-β1 expression in protocol kidney graft biopsy in patients treated with different immunosuppressive regimens. Protocol kidney graft biopsies were carried out in 77 patients with stable graft function at 1 year after kidney transplantation, treated with a triple-drug regimen based on cyclosporin A (CyA; $n = 49$) or tacrolimus (TAC; $n = 28$). Morphological findings were assessed using the Banff 97 classification. TGF-β1 expression was analysed using immunochemistry, and semiquantitatively scored in different renal structures (total score 0–18). Clinical data were analysed at the time of biopsy, and 12 months thereafter. No significant relation was found between the used immunosuppressive regimen and the histomorphological picture in the graft. TGF-β1 expression within graft tissue was significantly higher in patients treated with CyA when compared with TAC (9.94 ± 4.2 vs. 5.0 ± 3.2; $P < 0.001$). Serum creatinine and glomerular filtration rate (GFR; Cockroft-Gault calculation) were comparable in both groups but, in the course of the next 12 months, GFR significantly decreased only in the CyA-treated group (from 1.03 ± 0.33 to 0.96 ± 0.37 ml/s) while not changing in the TAC-treated group. Patients treated with TAC had significantly lower diastolic blood pressure and serum cholesterol. The significantly lower TGF-β1 expression in 1-year protocol kidney graft biopsy in TAC-treated patients with stable renal function, and the different development of graft function in both groups suggest a possible benefit of TAC for long-term graft acceptance.

Introduction

Chronic allograft nephropathy (CAN), a slow and progressive process, has been suggested to be the most important cause of late kidney graft loss. The main histopathological features of CAN include vascular intimal hyperplasia, tubular atrophy, glomerulopathy and interstitial fibrosis [1]. Transforming growth factor (TGF)-β1 has been proposed to play an important role in fibrogenesis in chronic nephropathies including CAN [2].

Nevertheless, fibrosis is a nonspecific finding which may also be induced by drugs like cyclosporin A (CyA) [3]. The aim of this study was to compare clinical and laboratory data together with histomorphological and immunochemistry findings in groups of patients treated with different immunosuppressive regimens, and to follow these patients for another 12 months.

Patients and methods

A total of 77 patients (mean age 52.3 ± 12.8 years, 55 males and 22 females) with stable graft function (serum creatinine up to 280 μM), 12 months after kidney transplantation, were enrolled into the study, and followed throughout the next year. Seventy-four patients received their first kidney graft, four their second graft. Sixty-six

patients were transplanted from cadaveric, 11 patients from living donors. Patients were treated with triple-drug immunosuppression based on either CyA group ($n = 49$) or tacrolimus (TAC group; $n = 28$), in combination with mycophenolate mofetil or azathioprine, and steroids. All patients gave their informed consent prior to their inclusion in the study.

Protocol biopsy

At 1 year after transplantation (12.7 ± 1.5 months), a protocol kidney graft biopsy was performed under ultrasound guidance, using a 16-gauge gun. All patients gave their informed consent, and signed their agreement with the protocol biopsy. The Ethics Committee of the Institute for Clinical and Experimental Medicine in Prague approved the study protocol.

Histomorphology

Biopsy tissues were stained with haematoxylin and eosin, and scored according to the Banff 97 working classification [4].

Immunohistology

Immunohistology was carried out using antihuman TGF-β1 monoclonal antibody (Biosource Int., Camarillo, CA, USA), and immunostained slides were graded on the basis of a previously described scheme [5]. TGF-β1 expression was assessed on a blinded basis, and graded semiquantitatively (0–1–2+) at nine different sites of graft structure: glomerular epithelium and intraglomerular structure, arterial endothelium and intima, peritubular capillaries, proximal and distal tubuli, cell infiltrate and interstitium. The sum of TGF-β1 expression at the particular sites was then calculated for each sample.

Clinical and laboratory data

The following variables were recorded; receiver age and gender, body mass index (BMI), and blood pressure, serum creatinine, glomerular filtration rate (GFR) calculated by the Cockroft-Gault formula, 24-h proteinuria, plasma cholesterol and triglycerides, human leucocyte antigen (HLA)-DR mismatch, and rejection first year. Blood samples were taken on the day of protocol biopsy (month 12), and after 1 year of follow up (month 24).

Statistics

The Mann–Whitney or the *t*-test, and ANOVA with repeated measures were used.

Results

Overall, CAN was found to be present in 55 samples (71%). The most common finding was mild CAN grade I and moderate CAN grade II. A serious finding of CAN grade III was made in two samples. About 22 (29%) patients had a normal histological finding. There was no difference between the groups in the percentage of patients with normal histomorphology or particular grades of CAN. The mean total value of the TGF-β1 staining score was higher in the cyclosporin group than in the TAC group, and this difference reached a high statistical significance (Table 1). Such a difference was found at all of the assessed sites with one exception, the arterial intima. No significant correlation was observed between TGF-β1 tissue expression and CAN grade as well as between TGF-β1 expression and renal function, and between TGF-β1 expression and CyA or TAC blood levels in either group.

Both groups were comparable in respect to their mean age, gender distribution, donor age, HLA-DR mismatch, rejections first year and BMI (Table 2). At the time of biopsy (12 months after transplantation), patients treated with the TAC-based regimen had significantly lower systolic and diastolic blood pressure than those treated with CyA. Serum creatinine was lower, and GFR calculated by Cockroft-Gault formula was higher in the TAC group, compared with the CyA group, but the difference did not reach statistical significance as did not the difference in proteinuria. Significantly lower were the mean values of cholesterol and triglycerides in TAC-treated patients.

At 12 months after biopsy (month 24), the values of systolic and diastolic blood pressure were not changed substantially, and the difference between the groups remained statistically significant. In the CyA-treated group, GFR (Cockroft-Gault calculation) significantly decreased (from 1.03 ± 0.33 to 0.96 ± 0.37 ml/s). In the TAC-treated group, GFR did not significantly change (1.10 ± 0.31 and 1.17 ± 0.35 ml/s), and the difference between the groups remained nonsignificant. Serum creatinine increased nonsignificantly in the CyA group while

Table 1. Histomorphological and immunochemistry findings in the graft.

	CyA group	TAC group
n	49	28
CAN 0	15 (30.6)	8 (28.6)
CAN I	26 (53.1)	16 (57.1)
CAN II	6 (12.2)	4 (14.3)
CAN III	2 (4.1)	0
TGF-β1 expression	9.9 ± 4.2	5.0 ± 3.2***

Values within parentheses represent percentage.
***$P < 0.001$ vs. CyA.

Table 2. Clinical and laboratory parameters at the time of biopsy (month 12) and at 1 year after biopsy (M24).

	Group	Month 12	Month 24	Comparison CyA:TAC at M12	Comparison CyA M12:CyA M24 TAC M12:TAC M24	Comparison CyA:TAC at M24
N	CyA	49				
	TAC	28				
Age (years)	CyA	52.3 ± 12.8		NS		
	TAC	47.1 ± 12.8				
Gender (M/F)	CyA	29/20				
	TAC	20/8				
Donor age	CyA	39.9 ± 14.9		NS		
	TAC	44.3 ± 12.2				
HLA-DR mismatch	CyA	0.73 ± 0.6		NS		
	TAC	0.75 ± 0.7				
Patients with rejection first year	CyA	11		NS		
	TAC	11				
BMI	CyA	28.1 ± 4.8	28.0 ± 4.3	NS	NS	NS
	TAC	26.8 ± 4.3	27.3 ± 4.2		NS	
Systolic BP	CyA	139.2 ± 15.7	138.3 ± 17.7	*	NS	*
	TAC	132.2 ± 16.2	132.0 ± 20.5		NS	
Diastolic BP	CyA	84.2 ± 9.7	83.3 ± 16.0	*	NS	*
	TAC	80.0 ± 8.6	79.2 ± 9.8		NS	
S-cr (μm)	CyA	146.1 ± 46.2	161.9 ± 84.8	NS	NS	NS
	TAC	137.2 ± 33.4	131.8 ± 38.4		NS	
GFR (Cockroft-Gault, mL/s)	CyA	1.03 ± 0.33	0.96 ± 0.37	NS	*	NS
	TAC	1.10 ± 0.31	1.17 ± 0.35		NS	
Proteinuria (g/day)	CyA	0.40 ± 0.49	0.76 ± 1.45	NS	NS	NS
	TAC	0.32 ± 0.66	0.24 ± 0.23		NS	
Cholesterol	CyA	6.33 ± 1.19	5.89 ± 1.40	**	NS	**
	TAC	5.27 ± 1.02	5.23 ± 1.01		NS	
Triglycerides	CyA	2.59 ± 1.22	2.33 ± 1.36	*	**	*
	TAC	2.14 ± 1.16	1.66 ± 0.61		**	

*$P < 0.05$, **$P < 0.01$.

HLA, human leucocyte antigen; BMI, body mass index; BP, blood pressure; S-cr, serum creatinine; GFR, glomerular filtration rate; CyA, cyclosporin A; TAC, tacrolimus.

decreasing in the TAC group. A similar trend was found in proteinuria, increasing nonsignificantly in the CyA group while decreasing in the TAC group. Cholesterol and triglycerides decreased nonsignificantly in both groups, but the difference between the groups remained significant (Table 2).

Discussion

Data of the prevalence of CAN after transplantation may vary broadly. While, in one group of patients, CAN was observed in 19% at 1 year [6], it was found in 25% of patients in another group [7] as early as 3 months after transplantation, and in 50% at 2 years after transplantation. In a group of patients assessed using chronic allograft damage index (CADI), the most common sign of CAN (fibrosis) was present in 85% of grafts at 1 year [8]. These data are similar to the prevalence of CAN in our patients (71% at 1 year after transplantation).

In our study, TGF-β1 expression within the graft was significantly higher in patients treated with a cyclosporin-based regimen than in patients treated with a TAC-based regimen. This difference was observed already in patients after kidney transplantation [9,10]. In those studies, however, kidney biopsies were performed due to deterioration of kidney graft function while, in our study, the biopsies were performed on a regular basis. In liver transplant patients, the difference in TGF-β1 expression was found as early as day 6 after transplantation [11]. Our findings are thus consistent with these observations. On the contrary, the prevalence of CAN was very similar in both groups, and did not indicate a different effect of CyA and TAC on histomorphological findings. It is possible that the histomorphological changes appear with a delay after TGF-β1 expression. Our study was not designed to demonstrate this, but the development of markers of renal function (serum creatinine, GFR) does suggest such a possibility.

At the time of biopsy, kidney graft function was similar in both groups. However, GFR significantly decreased in CyA-treated patients while remaining unaltered in the TAC-treated group.

In a recent prospective, randomized trial, such a difference in the serum creatinine values between TAC- and CyA-treated patients at 2 years after transplantation was statistically significant [12]. Our data seem to be in line with that observation. As we did not perform repeat protocol biopsy at 24 months after transplantation, we are not able to speculate about the effect of TGF-β1 on the development of fibrosis.

The significantly lower systolic and diastolic blood pressure as well as plasma lipids noted in TAC-treated patients are consistent with data from prospective randomized studies [13–15]. It is generally recognized that the lower blood pressure and lower plasma lipids are of benefit for the long-term kidney graft function [16]. Therefore, we assume that the better renal function observed in our TAC-treated patients at 1 year after graft biopsy may be in line with this hypothesis regardless the fact that we did not repeat our morphological evaluation.

In conclusion, a significantly lower TGF-β1 expression in 1-year protocol kidney graft biopsy in TAC-treated patients with stable renal function, and different development of graft function in both groups suggest a possible benefit of TAC for long-term graft acceptance.

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