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Clinocopathological evaluation in non-episode biopsies of renal transplant allograft

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Abstract Histopathological findings in renal allograft with stable function remain unclear. We therefore performed non-episode biopsy in the long-surviving renal allograft to investigate the histopathological changes. Our data show that, although arteriolopathy is characteristic of drug-induced nephropathy, it is unrelated to dosage and concentration of cyclosporine or tacrolimus in non-episode biopsy. We evaluated therefore the clinicopathological findings of arteriolopathy in this study. Non-episode biopsy was defined as follows: as serum creatinine level lower than, 2.0 mg/dl and a urinary protein level lower than 500 mg/day. A total of 65 biopsy specimens were enrolled in this study as non-episode biopsy. Twenty-nine specimens revealed arteriolopathy. There were no statistically significant differences between arteriolopathy and dosage or concentration of cyclosporine or tacrolimus. Arteriolopathy in non-episode biopsy was related to time of biopsy, kidney age, hypertension, and hyperlipidemia, suggesting that it is important for graft survival to strictly control blood pressure and blood lipid level.

Key words Non-episode biopsy · Arteriolopathy · Cyclosporine · Tacrolimus · Renal transplantation

Introduction

Short-term recipient and graft-survival rate after renal transplantation have been improved with the introduction of cyclosporine (CsA)-based [1] and tacrolimus (FK506)-based regimens [2]. Clinically, the adverse effects of these two drugs are similar, one of the most serious problems being chronic nephrotoxicity, which affects the long-term survival rate of renal allografts. These changes are assessed by histopathological findings of arteriolopathy, such as hyalinosis or insudative change of arteriole. However, arteriolopathy findings are unrelated to the dosage and concentration of CsA or FK506 in long-surviving renal they have been insufficiently studied in the context of renal allograft with stable function. We therefore performed renal biopsies as "non-episode biopsy" in long-surviving renal allografts with stable function, and investigated the clinicopathological characteristics of these allografts. Particular attention was paid to findings of arteriolopathy, such as drug-induced nephropathy.

Materials and methods

Patients

Since 1994, 175 renal biopsies have been performed on long-surviving renal allografts (at least 6 months after transplantation) with informed consent, at Osaka University Hospital, Inoue Hospital and Osaka Seamen's Insurance Hospital. In these cases, we defined "non-episode biopsy" as follows:

1. No episode of increase of serum creatinine level had been recognized before biopsy.
2. Serum creatinine (S-Cr) level was lower than 2.0 mg/dl, and urinary protein was less than 500 mg/day at the time of biopsy.

A total of 65 of the 175 cases were enrolled in this study as non-episode biopsy.

Table 1 shows the characteristics of the patients in this study. Thirty-nine were immunosuppresed with a CsA-based and 25 with an FK506-based regimen. Only one patient was immunosuppresed with neither CsA nor FK506, the main immunosuppressant in this case being azathioprine (not shown). There were no significant differences between the CsA-treated group and the FK506-immunosuppresed group in recipient age, kidney age or S-Cr level. In this study, we added donor age at transplantation to the years after transplantation and defined it as "kidney age" – in other words, the real age of the renal allograft. In most Japanese renal transplant cases, transplantation is performed from a living renal donor, in many cases father or mother, so kidney age was higher than the average in Europe and elsewhere. Only with regard to time of biopsy were significant differences found between the two groups, but this was because FK506 was introduced later than CsA.

Biopsy

We used a Biopty gun (C.R. Bird Inc., Covington, Ga.) with a 16-G true-cut needle. All biopsies were performed under ultrasound guidance using aseptic technique and local anesthesia with 1% lidocaine. No significant complications were seen in any of the biopsies. All these biopsy specimens were stained with hematoxylin-eosin, periodic acid-Sciff, periodic acid-methenamine silver and Masson's trichrome reagent. In addition, immunofluorescence or immunohistochemical stain was performed. Adequacy was defined as a minimum of four glomeruli in each sample. The biopsy specimens were read by at least two observers.

Table 1 Clinical characteristics of patients at the time of biopsy (*FK506* tacrolimus, *CsA* cyclosporin, *Bx* biopsy, *S-CR* serum creatinine)

	Total	CsA group	FK506 group
N	65	39	25
Age (years)	37.9 ± 10.6	40.8 ± 9.3	33.9 ± 11.1
Kidney age (years)	56.4 ± 13.6	57.8 ± 13.6	54.4 ± 13.7
Male:female	42:23	24:15	17:8
Living:cadaver	56:9	31:8	24:1
Time of Bx (days)	1634 ± 1252	1884 ± 1265	1035 ± 673
S-Cr (mg/dl)	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.3

Table 2 Histopathological findings of non-episode biopsies (overlapping diagnoses) (*AH* arteriolar hyalinosis score, *BC* borderline change, *G1A* grade 1A acute rejection, *GN* glomerular nephritis)

	Total	CsA group	FK506 group
Arteriolopathy	29	19	10
AH 1	19	10	9
AH 2	10	9	1
Subclinical rejection	14	9	5
BC	10	5	5
G1A	4	4	0
Chronic allograft Nephropathy	10	5	4
GN	5	4	1
Almost normal	19	7	12

Statistical analysis

Values of the clinicopathological report are given as means ± SD. Differences between groups were determined by chi-square, Mann-Whitney's U test or Fisher's Protected Least Significant Difference.

Results

Histopathological findings

The histopathological findings of 65 non-episode biopsy specimens are shown in Table 2. As can be seen, 29 of them (about 44%) showed evidence of arteriolopathy, such as chronic nephrotoxicity (hyalinosis or insudative change), 19 cases of which were treated with CsA and 10 with FK506. Fourteen cases of subclinical rejection

Table 3 Clinical characteristics and arteriolopathy

	No findings	AH1	AH2	*P*
Drug dose (mg/day)				
CsA	185.0 ± 61.7	150.0 ± 69.7	150.0 ± 40.8	NS
FK506	4.2 ± 2.6	3.5 ± 1.7	3	NS
Trough level (ng/ml)				
CsA	103.1 ± 39.6	96.0 ± 47.7	70.3 ± 35.3	NS
FK506	5.5 ± 4.1	5.4 ± 3.9	2.5	NS
S-Cr (mg/dl)	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	NS

were seen, which were categorized according to the Banff 97 working classification [3]. Of these 14 cases, 10 were diagnosed as borderline change and 4 were classified as grade 1A acute rejection. We treated these subclinical rejection cases with an increase of baseline immunosuppressant or steroid pulse therapy. However, one case that received no treatment, allowing the rejection to progress, finally reached grade 2A and steroid-resistant acute rejection. Chronic allograft nephropathy was found in 15 cases. Glomerulonephritis was seen in five cases, all of which were diagnosed as IgA nephropathy with immunostaining. As can be seen, in spite of fulfilling the criteria of "non-episode biopsies", only 19 specimens – less than 30% of the total – revealed normal findings.

Arteriolopathy

Table 3 shows the correlation between arteriolopathy and clinical findings in non-episode biopsies. Arteriolar hyalinosis (AH) score was based on the quantitative criteria of arteriolar hyaline thickening in the Banff 97 classification. No correlation was found between arteriolopathy and higher dosage or concentration of the two drugs, CsA or FK506. On the contrary, dosage and trough level of the two drugs had a tendency to decrease in proportion to the severity of arteriolopathy. With reference to S-Cr level, no differences were seen among the groups. From these results, arteriolopathy found in non-episode biopsies seemed not to be associated with CsA or FK506. Other factors that could be expected to affect arteriolopathy were therefore evaluated.

Table 4 shows the correlation of arteriolopathy with time of biopsy and kidney age. With regard to time of biopsy there were statistically significant differences between no the group with and the group without arteriolopathy, and time of biopsy increased in proportion to the AH score. Between AH 0 and AH 1, and between AH 1 and AH 2, statistically significant differences were seen. With respect to kidney age, frequency and quality of arteriolopathy was related to kidney age, and a statistically significant difference was seen between the AH 0 and AH 2 groups. These results proved that donor age was more important for vascular change of renal allograft than recipient age.

Next, blood pressure and serum lipid levels were evaluated and the results are shown in Figs. 1 and 2. As shown in Fig. 1, all the cases were categorized by blood pressure at the biopsy point into three groups: normal blood pressure, borderline (90 < systolic pressure < 95, or 140 < diastolic pressure < 160), and hypertension (systolic pressure > 95, or diastolic pressure > 160). If the borderline group and the hypertension group are put together and defined as the "uncontrolled group", a statistically significant difference was seen between the normal and the uncontrolled groups with regard to frequency of arteriolopathy. However, no correlation was seen between severity of arteriolopathy and blood pressure (data not shown).

With respect to blood lipid level, both total cholesterol level and triglyceride level had a tendency to increase according to the severity of arteriolopathy. However, no statistically significant difference was found.

Discussion

We have been performing protocol biopsy at 1 h, 1 month and 1 year after renal transplantation since 1995. However, histopathological findings of stable functioning renal allograft remain unclear. So we performed non-episode biopsy, which is useful for diagnosis of protocol biopsy.

Several studies of protocol biopsy have been published so far, in which acute rejection [4, 5] and chronic allograft nephropathy [6–8] were evaluated. Only one report, by Benigni et al. [9], concerned histopathological evaluation of nephrotoxicity in protocol biopsy. In

Table 4 Arteriolopathy by time of biopsy and kidney age

	No findings	Arteriolopathy		*P*
		(AH 1)	(AH 2)	
Time of biopsy (days)	1233 ± 1077	2249 ± 1072 (1710 ± 1075)	(2720 ± 1468)	< 0.0001
Kidney age (years)	53.5 ± 14.2	64.1 ± 8.0 (55.9 ± 13.6)	(66.1 ± 6.6)	< 0.005

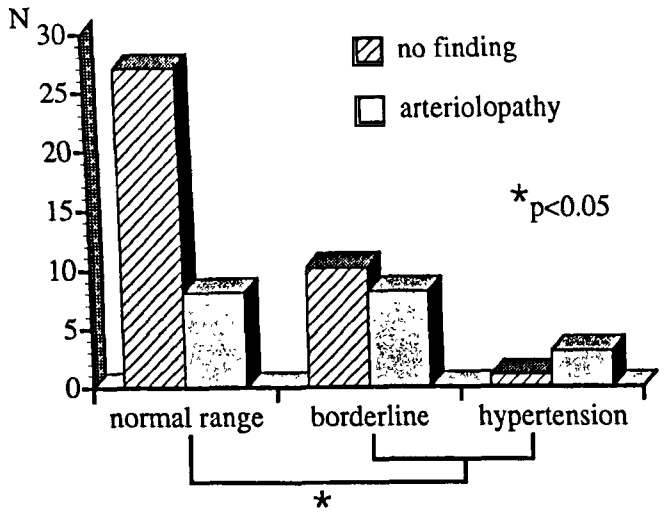

Fig. 1

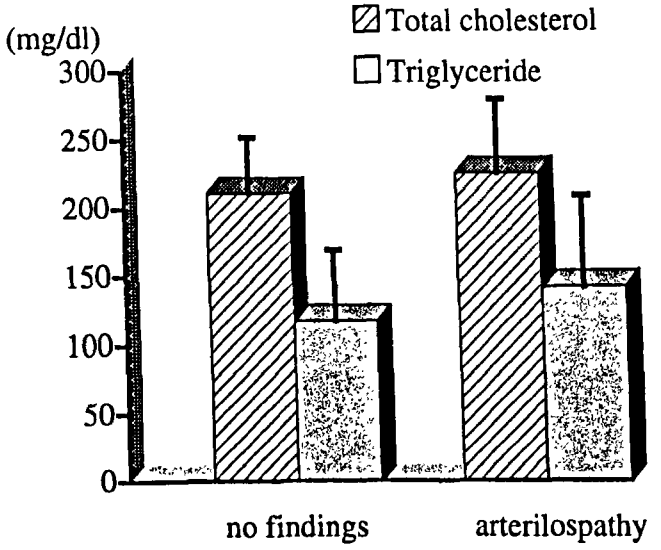

Fig. 2

their report, 10 of 22 patients (45.4%) had evidence of CsA-induced nephrotoxicity, and these results are comparable to our previous results [10]. The Concentration of cyclosporine was higher in the nephrotoxicity group than in the no lesion group in their report. However, as described above, in our non-episode biopsy cases, arteriolopathy was not associated with dosage or concentration of CsA or FK506, which induce nephrotoxicity. We therefore evaluated the clinicopathological findings of arteriolopathy in non-episode biopsies to clarify whether CsA and FK506 are responsible for arteriolopathy in non-episode biopsy.

As described above, arteriolopathy was related to kidney age, and donor age was more important than recipient age for graft survival. With regard to time of biopsy, there was a significant difference between the arteriolopathy group and the no findings group. From this result, one may hypothesize that the exposure period to CsA or FK506 may affect arteriolopathy. Thus, it is not necessarily the case that arteriolopathy in non-episode biopsy was not associated with CsA or FK506. However, arteriolopathy does not need to be treated with decrease of the drug dosage, because no episode of increase of S-Cr level was found in any of the cases of arteriolopathy 6 months after biopsy (data not shown).

Blood pressure clearly affects arteriolopathy, and serum lipid level seems also to be related to arteriolopathy. So the only precaution we can take against arteriolopathy is strict control of blood pressure and serum lipid level.

Since the first report of CsA-induced morphological alterations in the kidney [11], morphological findings and characteristics of CsA nephrotoxicity have been reported. CsA-associated arteriolopathy [12] is well known as the deposition of hyaline-like substances in lump form under the endothelial cells. FK506-associated arteriolopathy was reported to be similar to CsA-associated arteriolopathy [13]. Thus, there have been many reports about drug-induced arteriolopathy. From our experience, it is difficult to distinguish histopathologically CsA- or FK506-associated arteriolopathy from arteriolopathy due to aging, hypertension or hyperlipidemia. Where typical findings of hyalinosis in lump form are evident, these can be diagnosed as chronic nephrotoxicity, but as a matter of fact, most arteriolopathy cases in non-episode biopsy did not show typical findings of drug-induced arteriolopathy.

It is unclear whether arteriolopathy observed in stable functioning renal allograft progresses and deteriorates the renal function in long-term or not. To clarify this question, further observation regarding the clinical outcome of the patients who demonstrated arteriolopathy will be necessary.

Conclusions

1. In spite of lower dosage and concentration of the drugs, non-episode biopsy revealed arteriolopathy in about 44% of the patients
2. Arteriolopathy in non-episode biopsy was related to time of biopsy, kidney age, hypertension and hyperlipidemia.
3. Strict control of blood pressure and serum lipid level is important for graft survival.

References

1. The Canadian Multicentre Transplant Study Group (1983) A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N Engl J Med 309: 809–813
2. Gjertson DW, Cecka JM, Terasaki PI (1995) The relative effects of FK506 and cyclosporine on short- and long-term kidney graft survival. Transplantation 60: 1384–1388
3. Racusen LC, Solez K, Colvin RB, et al (1999) The Banff 97 working classification of renal allograft pathology. Kidney Int 55: 713–723
4. Rush DN, Jeffery JR, Gough J (1994) Sequential protocol biopsies in renal transplant patients. Transplantation 59: 511–514
5. Rush D, Jeffery J, Tropkov K, Solez K, Gough J (1996) Effect of subclinical rejection on renal allograft histology and function at 6 month. Transplant Proc 28: 494–495
6. Yilmaz S, Taskinen E, Häyry P, Isoniemi H (1996) Protocol core biopsy as intermediate efficacy end-point in chronic kidney allograft rejection. Transplant Proc 28: 491–493
7. Serón D, Moreso F, Bover J, et al (1997) Early protocol renal allograft biopsies and graft outcome. Kidney Int 51: 310–316
8. Solez K, Vincenti F, Filo RS (1998) Histopathological findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tacrolimus versus cyclosporine. Transplantation 66: 1736–1740
9. Benigni A, Bruzzi I, Marilena B, et al (1999) Nature and mediators of renal lesions in kidney transplant patients given cyclosporine for more than one year. Kidney Int 55: 674–685
10. Kyo M, Hatori M, Takahara S, et al (1998) Morphological findings in non-episode biopsies of kidney transplant allografts treated with FK506 or cyclosporine. Transplant Int 11 (Suppl 1):S100–S103
11. Myers BD, Ross J, Newton L, Leutscher J, Perlroth M (1984) Cyclosporine-associated chronic nephropathy. N Engl J Med 311: 699–705
12. Mihatch MJ, Thiel G, Basler V, Ryffel B, Landmann J, von Overbeck J, Zollinger HU (1985) Morphological patterns in cyclosporine-treated renal transplant recipients. Transplant Proc 17: 101
13. Japanese FK506 Study Group (1994) Morphological findings of renal allografts under FK506 therapy. Transplant Proc 26: 1933–1936