Angiotensin-converting-enzyme inhibitors slow renal decline in IgA nephropathy, independent of tubulointerstitial fibrosis at presentation

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Received 9 July 2004 and in revised form 19 January 2005

Summary

**Background:** Tubulointerstitial fibrosis (TIF) is a marker of progression of diabetic and non-diabetic nephropathy, correlating with creatinine clearance (CCr), and functional outcome. Angiotensin-converting-enzyme inhibitors (ACEIs) slow the rate of decline of renal function in proteinuric patients.

**Aim:** To examine whether ACEIs affect TIF, directly or indirectly.

**Design:** Prospective 3-year follow-up study.

**Methods:** We enrolled 49 patients with IgA nephropathy (IgAN), treating some with ACE inhibitors (\(n = 26\), 1–2 mg/day temocapril ortrandolapril) and some with calcium-channel blockers (CCB, \(n = 23\), 2.5–5 mg/day amlodipine). Blood pressure, serum creatinine, and urinalysis were measured monthly, and 24-h endogenous creatinine clearance (CCr) at least once a year.

**Results:** In the CCB group, TIF was positively correlated with the rate of decline in CCr (dCCr), consistent with previous observations. In the ACEI group, dCCr was lower (0.02 ± 0.02 vs. 0.06 ± 0.03), and the TIF-dCCr correlation was absent.

**Discussion:** In the absence of post-treatment histological data, it is not possible to say whether ACEIs have an effect on TIF. However, ACEIs appear to slow the progression of renal failure in IgAN, regardless of the degree of TIF at presentation.

Introduction

In the last decade, much interest has focused on renal fibrosis in the progression of renal diseases.\(^1\)–\(^3\) Quantitative evaluation of tubulointerstitial fibrosis (TIF) in the kidney can be the best predictor of final outcome of a variety of primary and secondary renal diseases.\(^4\)–\(^6\) Data from the AIPRI (Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency) trial and the REIN (The Raminipril Efficacy In Nephropathy) study show that angiotensin-converting-enzyme inhibitors (ACEIs) can significantly slow the rate of decline in renal function, in patients with non-diabetic glomerulopathy.\(^7\),\(^8\) In a recent review based on a series of studies including the REIN study, Remuzzi et al. concluded that urinary protein is one of the main mediators of glomerular damage to the tubulointerstitium, and that an ACEI, ramipril, can confer renoprotection via a reduction in urinary protein excretion in progressive, non-diabetic, proteinuric patients.\(^9\) ACEIs have also been shown to slow the progression of nephropathy in type 1 diabetes patients,\(^10\) but the effects of ACE inhibition in type 2 diabetes remain inconclusive, possibly due to its heterogeneous nature.\(^11\) Since TIF has a significant impact on the prognosis of a variety of renal diseases, the results from these studies suggest that...
ACEIs may have direct and/or indirect effects on TIF in non-diabetic and diabetic renal diseases. Although a number of animal studies suggest that the renin-angiotensin-aldosterone-system (RAS) involves TIF, few published clinical observations support this hypothesis. Some recent studies have reported the efficacy of ACEIs in IgA nephropathy (IgAN), but it remains unknown whether the effects of ACEIs on IgAN are mainly by reducing proteinuria, or by other mechanisms. We conducted a prospective study to determine how an ACEI affected the functional prognosis of patients with biopsy-proven IgA nephropathy (IgAN), as a function of the degree of TIF.

Methods

Patients

After obtaining informed consents, 49 Japanese patients with IgAN were enrolled in the study at Saitama Medical School Hospital, and Saitama Social Insurance Hospital, when they were about to undergo renal biopsy. Histopathological diagnosis of IgAN was based on the demonstration of prominent IgA deposition in the mesangial area by immunofluorescence. Patients with diabetes mellitus, hyperlipidaemia, liver diseases, systemic lupus erythematosus, and Henoch-Schoenlein purpura were excluded. Patients who had a past history of treatment with ACEIs or steroids were also excluded. Enrolled patients randomly assigned to the ACEI group (n = 26) were administered 1–2 mg/day of temocapril or trandolapril, and those in the calcium channel blocker (CCB) group (n = 23) were administered 2.5–5 mg/day of amlo-dipine. The target blood pressure in the outpatient clinic was 130/85 mmHg, and was achieved by the administration of these antihypertensives, with an additional 10–20 mg/day of arotinolol when needed. The patients, whose blood pressures were below 130/85 mmHg at registration, received a minimum dose of each agent. Blood pressure, serum levels of creatinine, and urinalysis were measured on a monthly basis for at least 3 years. We measured 24-h endogenous creatinine clearance (CCr) at least once a year. The index of the decline in CCr over 3 years (dCCr index) was calculated as (initial CCr–final CCr)/initial CCr.

Quantification of TIF

MT-stained kidney sections were examined under a light microscope connected to a digital CCD camera (HC-2500, Fuji Film Company). Whole sections were scanned at 100x, and non-overlapping fields were saved as graphic image files. Images were analysed quantitatively with image analysis software (MacScope, Mitani). As the blue-stained collagenous area in MT stain was used as an index of fibrosis in each image, TIF index was calculated as (collagenous area in blue/non-glomerular and arterial total area).

Statistics

Data were expressed as mean±SD. ANOVA and Bonferroni/Dunnett’s test was used to determine the significance of difference in multiple comparisons. Spearman correlation coefficients (two-tailed) were used to evaluate whether dCCr index was correlated with TIF index. Values of p<0.05 were considered statistically significant.

Results

Clinical and pathological data

All enrolled patients completed the study, and the baseline and the final data of patients are shown in Table 1. Serum creatinine levels at the entry were 0.96–1.42 mg/dl. The two groups were comparable at baseline. Blood pressure was well controlled, and remained at or below the target level (130/85 mmHg) in both groups. Proteinuria slightly increased in the CCB group, and significantly decreased in the ACEI group. Although there was no significant difference in the level of serum creatinine between the two groups at the end of follow-up, CCr was slightly but significantly larger, and dCCr index significantly smaller in the ACEI group than in the CCB group (Table 1).

To evaluate TIF quantitatively, the fibrotic areas that stained blue in the MT-stained biopsy sections were counted using a computerized image analyser (Figures 1a, 1b), and the TIF index was calculated (Table 1). There was no significant difference in TIF index between the two groups.

Clinical and pathological correlations

Other parameters that changed from baseline in the two groups included TIF index, serum creatinine, CCr, and proteinuria, showing weak, positive correlations (Figure 2a). However, no correlation was observed between TIF index and age or blood pressure. In the longitudinal evaluation, dCCr index had a significant positive correlation with TIF index in the CCB group (r = 0.532, n = 23, p = 0.0125), but there was no correlation in the ACEI group (r = -0.372, n = 26, p = 0.0631) (Figure 2b). There was a significant difference...
between the regression lines of the two groups (p = 0.003).

**Discussion**

In this study, RAS blockade by ACEI slowed the rate of decline of renal function in IgAN patients, with or without TIF. Tubulointerstitial alterations preceding advanced TIF (scarring) consisted of interstitial monocytes infiltration, fibroblast proliferation, and extra-cellular matrix (ECM) protein accumulation; a number of studies suggest the involvement of Ang II in these alterations.

A hallmark of renal injury is excessive urinary protein excretion, which is not only an indicator of glomerular damage, but may contribute to tubulointerstitial alterations, regardless of the type of glomerular injury. Proteins filtered from the glomerulus are reabsorbed by the proximal tubular epithelial cells (PTECs). An excess load of proteins in PTECs may induce lysosomal rupture and phenotypic changes. These release or activate local vasoconstrictors such as Ang II and endothelin, chemotactic proteins such as MCP-1 and OPN, and profibrotic growth factors such as TGF-β1, leading to tubulointerstitial alterations. Thus RAS blockade by ACEI probably attenuates TIF via two different pathways. On the one hand, ACEIs may reduce urinary protein-induced Ang II generation and suppress subsequent increases in profibrotic factors induced by Ang II, as described above. On the other, antiproteinuric effects of ACEIs via their haemodynamic actions could reduce tubular exposure to proteins, providing an additional mechanism for attenuating TIF. Both TIF and proteinuria are good prognostic indicators in most of the progressive glomerulopathies, and in the AIPRI and REIN studies, renoprotection by ACEIs correlated with the degree of reduction in urinary protein excretion.

However, in the present study, we found only a weak negative correlation between the degree of reduction in urinary protein excretion and dCCr.
index (data not shown). This might be accounted for by a difference in the patients studied. In the AIPRI study and the REIN study, both groups were large, and included a wide variety of proteinuric renal diseases, and the most significant renoprotective effects were seen in patients with urinary protein excretion \( \geq 4 \) g/24 h, (measured as the degree of reduction in the amount of protein excreted). In our study, a relatively small number of patients with IgAN were enrolled, and few had urinary protein excretion \( > 2 \) g/24 h. Thus the direct effects of ACEIs on TIF might be more prominent in our study than in the REIN study.

In this study, we demonstrated that interstitial fibroblast-like cells were positive for AT1R in the IgAN kidneys. Like human renal fibroblasts, cultured rat renal fibroblasts (NRK 49F) also possess functional AT1aR, and produce TGF-\( \beta \) and fibronectin in response to Ang II. Renal fibroblasts derived from fibrosing kidneys often bear profibrotic phenotypes such as hyperproliferative growth, enhanced synthesis of collagen and production of profibrotic cytokines, compared with those derived from normal kidneys. In addition to these phenotypes, fibrosis-derived fibroblasts have enhanced profibrotic responsiveness to RAS, and this may account for our experience of superior effectiveness of ACEIs on IgAN patients with TIF.

This study has some limitations. First, changes in RAS were not evaluated by plasma renin activity or plasma aldosterone concentration, leaving some uncertainty about systemic RAS status. This is mainly because of the variation in these measurements with time, stature, meals, emotions, and so forth. Secondly, recently ACE gene polymorphisms have attracted attention as a predictor of efficacy of ACEIs in several renal diseases. Yoshida et al. found a high frequency of the DD genotype in IgAN with progressive renal deterioration, but subsequent studies have produced conflicting results. Schena et al. concluded in their meta-analysis that the development of IgAN was not associated with the presence of D allele in the Asian and Caucasian populations. Therefore, we did not determine ACE gene polymorphism of patients at enrolment. Thirdly, we lack pathological data after the treatment, because patients were unwilling to have a repeat biopsy. Therefore our conclusion are somewhat speculative, based on the changes in physiological data, and the pathological findings at the start of the study. Lastly, the allocation of patients to treatment groups was not random, and this may have introduced some bias.

In conclusion, ACEIs retarded the progression of IgAN in kidneys with or without TIF. Co-administration of ACEIs and ARB additively reduced urinary protein excretion in these patients, as in the COOPERATE study, in which drug combination therapy retarded progression of non-diabetic renal diseases more efficaciously than either monotherapy. This suggests that inhibition of RAS at multiple points is a promising approach to retard the progression of diabetic and non-diabetic renal diseases, and should be investigated using prospective clinical trials.

Acknowledgements
A part of this study was presented at the 34th annual meeting of American Society of Nephrology (San Francisco, USA, 2001).
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