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Original Paper

ACE Inhibition in Anti-Thy1 Glomerulonephritis Limits Proteinuria but Does Not Improve Renal Function and Structural Remodeling

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Key Words
Anti-Thy1 glomerulonephritis · Angiotensin II · ACE inhibitor · Glomerulosclerosis

Abstract
Background/Aims: ACE inhibitor (ACE-I) treatment effectively inhibits proteinuria and ameliorates the course of various renal diseases. In experimental glomerulonephritis, however, angiotensin II (AngII) infusion has also been shown to be renoprotective. We evaluated the long-term (28 days) course of anti-Thy1 glomerulonephritis in animals with suppressed AngII formation by ACE-I treatment. ***Methods:*** Brown Norway rats received perindopril (2.8 mg/kg/day, n = 12), dihydropyridine calcium-antagonist amlodipine (Ca-A; 13 mg/kg/day, n = 6) or were left untreated (n = 14). All animals were monitored for blood pressure, proteinuria, and creatinine clearance after anti-Thy1 injection. Renal histology was assessed at day 7 and 28. ***Results:*** Systolic blood pressure was equally reduced by ACE-I and Ca-A treatment. AngII suppression prevented development of proteinuria, but did not protect against glomerular microaneurysm formation or reduction in creatinine clearance. After resolution of the microaneurysms, animals with suppressed AngII production showed a modest increase in glomerulosclerosis and vasculopathic thickening of intrarenal vessels. ***Conclusions:*** In anti-Thy1 glomerulonephritis, suppression of AngII formation does not protect against the induction of glomerular damage and is associated

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with mild aggravation of adverse renal fibrotic remodeling. Proteinuria, however, is effectively prevented by ACE-I treatment. Ca-A treatment did not affect the course of glomerulonephritis, indicating that ACE-I effects are blood pressure independent.

Introduction

The anti-Thy1 glomerulonephritis model is characterized by marked transient proteinuria and involves both renal injury and repair. It therefore offers the potential to study various components of renal disease. Thy1 is expressed on glomerular mesangial cells. Binding of the anti-Thy1 antibody delivered by single injection results in complement-mediated mesangiolysis with secondary endothelial damage [1, 2]. The model is self-limiting and fully reversible in healthy animals, while uninephrectomized rats injected with anti-Thy1.1 antibody develop progressive glomerulosclerosis [3].

The potent antiproteinuric effects of ACE inhibitors (ACE-I) are well established. Angiotensin II (AngII) is a pathogenic factor in various renal diseases. However, AngII may also have renoprotective effects in early stages of kidney disease development. Stimulation of renal angiogenesis by AngII occurs during physiological postnatal kidney development [4] and drives the accelerated glomerular recovery seen after infusion of AngII during early-phase anti-Thy1 glomerulonephritis [5, 6]. Consistently, glomerular endothelial cell proliferation after anti-Thy1 glomerulonephritis was inhibited with AngII receptor blockade [7]. The attenuated glomerular injury with AngII infusion is in apparent contrast with previous studies in the anti-Thy1 model suggesting attenuation of renal damage by treatment with ACE-I or AngII receptor blocker [7–13]. The latter studies reported improvement mainly in terms of reduced proteinuria and early reduction of matrix expansion. We hypothesized that the suppression of AngII formation would not improve late-stage renal function in anti-Thy1 glomerulonephritis despite possible antiproteinuric effects. Therefore, we evaluated the effect of ACE-I treatment using perindopril on proteinuria, creatinine clearance, induction of glomerular damage and renal histological recovery in nephritic rats. To dissociate direct effects of ACE-I treatment from those on blood pressure reduction, we also included nephritic rats treated with the dihydropyridine calcium-antagonist amlodipine (Ca-A) in our study.

Animals and Methods

Animals

Male 11-week-old Brown Norway/RijHsd (BN) rats weighing 280–300 g (Harlan, Horst, The Netherlands) housed in a 12/12 h light/dark cycle and receiving food and acidified water ad libitum were used for all experiments. The Animal Ethics Committee of our institution approved all protocols.

Experimental Design

Anti-rat Thy1.1 monoclonal antibody (ER4, 1 mg/kg body weight) was injected intravenously on day 0 in all rats. Rats treated with ACE-I perindopril (gift from Servier; 33 mg/l in the drinking water, resulting in an average dose of 2.8 mg/kg body weight/day; n = 12) since day 3 before anti-Thy1 injection were compared to controls (n = 14) and rats treated with Ca-A (gift from Servier; 150 mg/l in the drinking water, resulting in an average dose of 13 mg/kg body weight/day; n = 6). Rats were placed in metabolic cages with free access to food

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and water at several time points to collect 24-hour urine samples. Systolic blood pressure was measured in conscious rats by tail-cuff sphygmomanometry (IITC, San Diego, Calif., USA). Rats were sacrificed at day 7 (n = 4 control vs. n = 6 ACE-I-treated animals) and day 28 (n = 10 control vs. n = 6 ACE-I-treated and n = 6 Ca-A-treated animals), and kidneys were excised after perfusion at 120 mm Hg with ice-cold 0.9% saline. Kidney specimens were transversely cut in 1-mm slices, fixed in 4% buffered formaldehyde and embedded in paraffin.

Measurements of Renal Function Parameters

Urinary protein concentration was determined by Bio-Rad Protein assay (Bio-Rad Laboratories GmbH, München, Germany) in 24-hour urine samples. Plasma and urinary creatinine levels were determined colorimetrically (Sigma Diagnostics Inc., St. Louis, Mo., USA). The creatinine clearance, calculated by the standard formula, was used as an estimate of glomerular filtration rate (GFR).

Renal Histology

Renal morphology was evaluated using Periodic Acid Schiff (PAS)-stained 5-μm paraffin sections, based on at least 50 glomeruli per kidney section. The percentage of glomeruli containing microaneurysms was counted in sections from rats terminated at day 7. The Glomerular Sclerosis Index (GSI) was calculated as previously described [14] in sections from rats terminated at day 28. For the GSI, glomeruli were scored based on the percentage of the glomerular area that was sclerotic (normal = 0, <25% = 1, 25–50% = 2, 50–75% = 3, 75–100% = 4). Renal artery wall thickness of the cortical intrarenal arteries in PAS-stained kidney sections was measured using AnalySIS 3.0 software (Soft Imaging Systems, Münster, Germany), based on lamina externa and interna diameters and using the average of at least five vessels per kidney section.

Statistical Analysis

All values are expressed as mean ± SEM. Data were analyzed using SPSS version 11.0 software. Differences between groups were analyzed using the Student t test, and one- or two-way ANOVA with Kruskal-Wallis post-hoc test if appropriate. A p value <0.05 was considered significant.

Results

ACE-I and Ca-A Lowered Systolic Blood Pressure

Baseline systolic blood pressure was 112 ± 3 mm Hg. At the time of anti-Thy1 injection, systolic blood pressure was reduced by 19 ± 2 mm Hg in ACE-I-treated rats ($p < 0.001$) and by 22 ± 3 mm Hg in Ca-A-treated rats ($p = 0.001$), and this level of blood pressure reduction was sustained. The difference in blood pressure reduction for Ca-A and ACE-I treatment was not significant.

ACE-I, but Not Ca-A, Inhibited the Development of Proteinuria after Anti-Thy1 Injection

In control rats, induction of glomerulonephritis caused an increase in urinary protein excretion peaking at day 7 (fig. 1). ACE-I treatment significantly reduced the development of proteinuria and resulted in normalization of proteinuria at day 14 when proteinuria was still high in controls ($p < 0.01$ for treatment interaction in two-way ANOVA for time and treatment interaction). Ca-A treatment did not affect the development of proteinuria (fig. 1).

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Table 1. Biochemical parameters of renal function

	Non-nephritic controls	aThy-1-nephritic controls		aThy-1-nephritic + perindopril		aThy-1-nephritic + amlodipine	
		day 7	day 28	day 7	day 28	day 7	day 28
Plasma urea, mM	8.8 ± 1.0	10.4 ± 0.9	8.9 ± 0.4	16.5 ± 0.8*, #	17.0 ± 1.6*, #	8.5 ± 0.4	7.8 ± 0.4
Creatinine clearance ml/min/kg BW	7.5 ± 1.2	7.9 ± 1.7	4.7 ± 0.4*	5.9 ± 0.4	4.1 ± 0.5*	9.6 ± 0.7	5.5 ± 0.5
Proteinuria, mg/24 h	13.1 ± 0.5	68.5 ± 10.7*	20.6 ± 3.2*	24.3 ± 3.2*, #	11.0 ± 1.1*, #	58.1 ± 5.6*	15.5 ± 0.9

* $p < 0.05$ compared to non-nephritic controls; # $p < 0.05$ compared to nephritic controls at the corresponding time point.

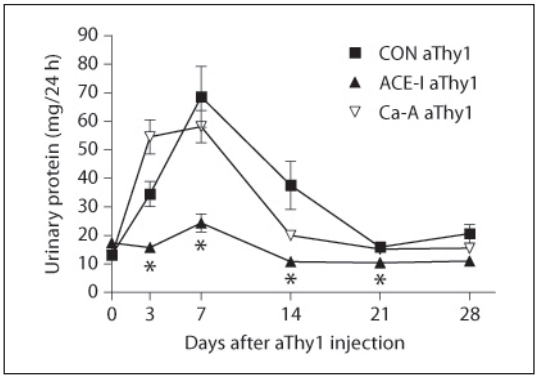

Fig. 1. Development of proteinuria over the course of anti-Thy1 glomerulonephritis. Induction of anti-Thy1 glomerulonephritis caused a transient rise in proteinuria in untreated rats (CON aThy1), which was not affected by treatment with calcium-antagonist amlodipine (Ca-A aThy1), but nearly fully prevented by treatment with the ACE inhibitor perindopril (ACE-I aThy1). * $p < 0.05$ compared to untreated controls.

ACE-I Did Not Attenuate the Reduction of Creatinine Clearance after Anti-Thy1 Glomerulonephritis

Creatinine clearance was reduced at day 28 in all nephritic rats (table 1). In perindopril-treated animals, creatinine clearance was not beneficially affected.

ACE-I Does Not Prevent Microaneurysm Formation in Early-Phase Anti-Thy1 Glomerulonephritis

Consistent with previous reports [2, 15, 16], injection of anti-Thy1 caused acute mesangiolysis, capillary ballooning and microaneurysm formation at day 7 (fig. 2). ACE-I treatment did not affect the percentage of microaneurysmatic glomeruli (40 ± 4 vs. 41 ± 5% in controls; p = not significant) at that time point. At day 28, microaneurysms were only sporadically observed in sections from both treated and untreated nephritic animals.

ACE-I Treatment Aggravated Adverse Fibrotic Remodelling during Late-Stage Anti-Thy1 Glomerulonephritis

At day 28, nearly all microaneurysms had resolved, but around one third of glomeruli showed segmental glomerular sclerosis (fig. 2). At day 28, ACE-I-treated animals had a slightly higher average glomerulosclerosis score than controls, which was statistically significant (GSI 0.49 ± 0.07 vs. 0.36 ± 0.03, $p < 0.05$; fig. 3). To further evaluate possible adverse fibrotic remodeling, we assessed intrarenal intima-media thickness as this was pathologically increased with ACE inhibition in experimental kidney transplantation [17]. Indeed also in our model, ACE-I treatment was associated with signs of renal artery vasculopathy, with

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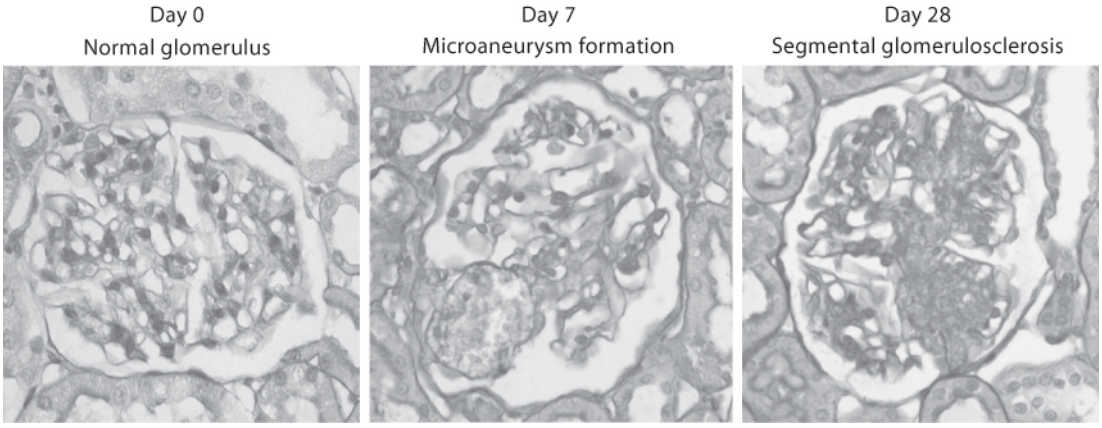

Fig. 2. Morphological features of the anti-Thy1 glomerulonephritis model. Injection of anti-Thy1 in untreated rats causes acute mesangiolysis and capillary ballooning, which is visible on PAS-stained sections at day 7 as microaneurysms have formed. At day 28, nearly all microaneurysms are resolved, but many glomeruli show segmental glomerular sclerosis.

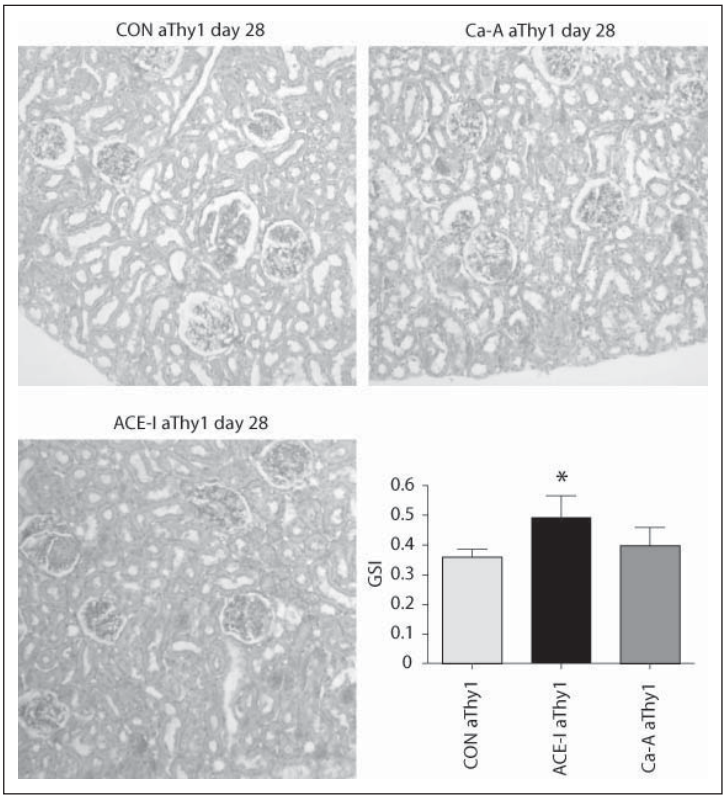

Fig. 3. Glomerulosclerosis during late-phase anti-Thy1 glomerulonephritis. In kidney sections of rats with late-phase anti-Thy1 glomerulonephritis, a proportion of glomeruli showed signs of segmental glomerulosclerosis, which was histologically quantified using the GSI (see Animals and Methods section for specification of the scoring procedure). The GSI was higher in ACE inhibitor perindopril-treated animals (ACE-I aThy1) than in untreated controls (CON aThy1). Treatment with calcium-antagonist amlodipine (Ca-A aThy1) did not affect the development of glomerulosclerosis. * $p < 0.05$ compared to untreated controls.

a significantly increased wall thickness of the cortical arteries at day 28 (intima-media surface area 4,046 ± 419 vs. 2,689 ± 195 μm^2, $p < 0.05$; fig. 4). GSI in Ca-A-treated animals did not differ from controls (0.39 ± 0.06 vs. 0.36 ± 0.03, p = not significant; fig. 3), and cortical artery wall thickness was unchanged in untreated controls and Ca-A-treated animals at day 28 (fig. 4).

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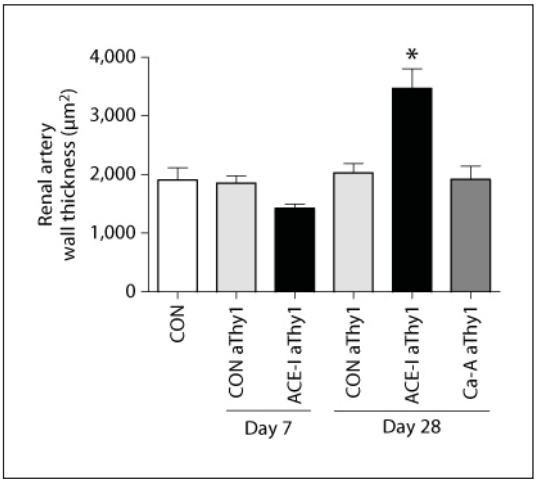

Fig. 4. Renal artery vessel wall thickness. Compared to untreated rats (CON aThy1), treatment with ACE inhibitor perindopril (ACE-I aThy1) was associated with vasculopathic thickening of the blood vessels in the renal cortex. Calcium-antagonist amlodipine (Ca-A aThy1) did not affect intrarenal vessel morphology. * $p < 0.05$ compared to untreated controls at the equal time point.

Discussion

A single intravenous injection of anti-Thy1 antibody is known to cause reversible glomerulonephritis characterized by marked transient proteinuria and multiple stages of structural glomerular pathology. Here, we show that ACE-I treatment during anti-Thy1 glomerulonephritis effectively reduces the development of proteinuria, but does not protect against the induction of glomerular damage or decline in renal function. ACE-I treatment could not prevent glomerular microaneurysm formation and was even associated with a modest but statistically significant accentuation of glomerulosclerosis and vasculopathic thickening of intrarenal vessels. Our study indicates that the multiple pathophysiological components during the course of anti-Thy1 glomerulonephritis are differentially affected by the suppression of AngII formation.

We show a potent protective effect of ACE inhibition on the proteinuria occurring during the development of glomerular damage. Several previous studies have similarly shown a reduction in proteinuria during anti-Thy1 glomerulonephritis by treatment with ACE-I or AngII receptor blockers [7–13]. Consistently, proteinuria tended to stay elevated for a longer period of time when rats with anti-Thy1 glomerulonephritis received AngII infusion [6]. Proteinuria is a practical and powerful indicator of the course of renal disease [18]. Preventing or reducing proteinuria by ACE-I is well-established and likely to make a major contribution to the beneficial effects of ACE-I on the course of renal disease. Proteinuria is thought to not only reflect renal damage, but also play a pathophysiological role in the progression of renal damage itself [19]. Indeed, in the aforementioned studies of ACE inhibition in anti-Thy1 glomerulonephritis, early matrix remodelling was inhibited. In our study, glomerular microaneurysm formation was not reduced by ACE inhibition. As glomerular microaneurysm formation reflects the degree of endothelial damage, the antiproteinuric effects do not appear to be attributable to endothelial protection but may be mediated through another mechanism. Such a specific antiproteinuric effect of ACE-I treatment may be explained by direct effects on the selective properties of the glomerular membrane size [20] independent of the effects on the glomerulonephritic disease process.

Our study shows that in anti-Thy1 glomerulonephritis, inhibition of AngII formation promoted late-stage adverse sclerotic remodelling in both the glomerulus and intrarenal vasculature. This is in line with previous reports indicating that AngII infusion accelerated glomerular endothelial recovery [6], reduced influx of inflammatory cells, reduced mesangial

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proliferation, and limited matrix production [5]. Similar deterioration of renal function and aggravated graft vasculopathy was observed in association with ACE-I treatment in the Fisher-to-Lewis experimental rat model of chronic renal transplant failure, even though the development of proteinuria and glomerulosclerosis was prevented [17]. Furthermore, in both healthy and adriamycin-induced proteinuric rats on a low-sodium diet, ACE-I treatment caused interstitial renal damage and vasculopathic hypertrophy of intrarenal arteries [21].

We did not observe any effect of dihydropyridine Ca-A treatment on the course of anti-Thy1 nephritis. Unlike ACE-I, Ca-A treatment did not adversely affect the disease course, despite giving comparable blood pressure reduction. Thus, the adverse effects observed with ACE-I treatment appear to be independent of the systemic blood pressure-reducing action. Nevertheless, we cannot exclude a role for altered renal blood flow and filtration pressure, which are affected differently by dihydropyridine Ca-A and ACE-I [22].

In conclusion, our study suggests that ACE inhibition is effective in reducing proteinuria, but does not beneficially affect other pathological components involved in anti-Thy1 glomerulonephritis. This is in line with the concept that AngII may have specific renoprotective effects [23, 24]. Also, these results suggest that in specific conditions, proteinuria may thus not be a reliable indicator of the course of renal disease treated with ACE inhibitors.

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