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Original Paper

Add-On Effect of Angiotensin Receptor Blockade (Candesartan) on Clinical Remission in Active IgA Nephropathy Patients Treated with Steroid Pulse Therapy and Tonsillectomy: a Randomized, Parallel-Group Comparison Trial

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Key Words
Angiotensin receptor inhibitor • Clinical remission • IgA nephropathy • Steroid pulse therapy • Tonsillectomy

Abstract
Background/Aims: Angiotensin receptor blockers (ARBs) may be beneficial for clinical remission during conventional therapy with tonsillectomy and steroid pulse (TSP) for active IgA nephropathy. ***Methods:*** Seventy-seven patients with active IgA nephropathy were randomly assigned to the control arm with conventional regimen (TSP followed by oral prednisolone) (n = 37) or the ARB arm with conventional regimen plus ARB candesartan for the first 6 months (n = 40). Patients not achieving proteinuria remission at 12 months in either arm were administered candesartan, which was titrated until the 24-month follow-up. The primary endpoints were remission of proteinuria (<0.3 g/gCr) and hematuria at 12 months. ***Results:*** Baseline proteinuria (g/g Cr) were comparable between the the control and ARB arm (1.02 vs.

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0.97, P = 0.97). Similarly, cumulative remission rates at 6, 12, and 24 months were comparable between the control and ARB arms (37.8% vs. 35% [*P* = 0.80], 48.7% vs. 38.5% [*P* = 0.37], 71.4% vs. 51.3% [*P* = 0.08]). Proteinuria, which was slightly worse in the control arm than in the ARB arm at 6 months, was comparable afterwards (0.20 vs. 0.23 g/g Cr at 12 months; 0.12 vs. 0.13 g/g Cr at 24 months). Significant reductions observed in urinary angiotensinogen were almost comparable between the two treatment arms at both 6 and 12 months. ***Conclusion:*** Early candesartan treatment combined with TSP may not benefit clinical remission regardless of the blood pressure. ARB titration later during the treatment might provide benefit for patients with active IgA nephropathy.

Introduction

IgA nephropathy is the most common primary glomerulonephritis worldwide, especially in Asia including Japan [1, 2]. Among primary glomerular diseases, IgA nephropathy is one of the leading causes of end-stage renal disease (ESRD)[3]. However, established therapies to prevent the progression of IgA nephropathy to ESRD are limited [4-6]. Clinical remission, defined as the resolution of proteinuria and hematuria, was shown to associate with favorable long-term outcomes [7-9]. Studies revealed that the contribution of glomerular capillary inflammation and glomerular hemodynamic abnormalities, two major pathogenic processes involved in IgA nephropathy, to the development of renal damage varied during the disease course [10]. Findings from recent basic and clinical studies suggest a fundamental role for abnormal mucosal immunity in the development of glomerular inflammation [11, 12]. The potential efficacy of tonsillectomy also supports the presence of a link between mucosal immunity and progression of IgA nephropathy [13, 14]. Randomized control trials as well as observational studies suggest that combination therapy with tonsillectomy and steroid pulse therapy (TSP) might promote clinical remission [7, 14, 15]. Although TSP has been widely used in patients with IgA nephropathy in Japan [16], the reported remission rates vary widely [10, 16, 17]. Since clinical remission might potentially delay the progression of IgA nephropathy [7, 8], improving remission rates by TSP might promote favorable renal outcomes. However, factors contributing to improved remission rates remain unclear.

Among the proposed pathogenic processes underlying IgA nephropathy [4, 18], glomerular inflammation and the activation of the renin-angiotensin system (RAS), especially in the kidney, appear to be major contributors [19-21]. Resolution of glomerulonephritis by treatment approaches that can complement TSP might be crucial for clinical remission. For example, self-resolution of glomerular inflammation was shown in Thy1 nephritis, a model of mesangial proliferative glomerulonephritis [22]. However, concurrent nephrectomy has the potential to redirect self-resolution into a progressive phenotype in which glomerulonephritis might develop [22]. Studies showing augmentation of renal RAS in nephrectomized animals [23] as well as in patients with IgA nephropathy [21, 24] suggest that renal RAS might be responsible for both glomerular hypertension and glomerular inflammation. Moreover, RAS is well known to activate inflammatory processes [25] and vice versa [26], leading to a vicious cycle. In support of the potential role of RAS activation in nephropathy, angiotensin receptor blockers (ARBs) were shown to attenuate crescent formation [27].

We hypothesized that RAS inhibition, independent from its role in blood pressure regulation, might improve the efficacy of TSP in achieving clinical remission by blocking renal RAS and alleviating secondary glomerular hypertension. In this randomized, open-labeled control study, we evaluated the effects of short-term combination treatment with the ARB candesartan and TSP in patients with active IgA nephropathy.

Materials and Methods

Study Design

This open-label, and randomized clinical trial (ACTRN12610000516088) was conducted at three institutions, including one university and two community hospitals, in Okinawa, Japan. The study protocol was approved by the institutional review board of the University of the Ryukyus and was conducted in accordance with the Declaration of Helsinki and national guidelines; furthermore, all patients provided written informed consent.

Patients

Patients between the ages of 15 and 70 years with histologically proven IgA nephropathy were eligible if they fulfilled the following inclusion criteria: urinary protein ≥0.5 g/gCr; serum creatinine ≤1.5 mg/dL; and active glomerular lesions defined by the presence of necrosis, crescent formation, or urinary red blood cell count ≥10/high-power field (HPF) for a minimum of 3 months. The exclusion criteria were as follows: contraindications to ARBs, treatment with a RAS inhibitor within 4 weeks before enrollment, diabetes mellitus, severe hypertension defined as ≥200 mmHg systolic blood pressure or ≥100 mmHg diastolic blood pressure, and recent onset of cardiovascular disease. Treatment was discontinued 4 weeks before enrollment and after obtaining informed consent in patients taking RAS inhibitors.

Comorbidities in this analysis were defined as follows. Hypertension was defined as >2 ambulatory blood pressure of ≥140 mmHg systolic and/or 90 mm Hg diastolic, or treatment with antihypertensive agents. Diabetes mellitus was defined as ≥2 fasting plasma glucose level measurements of ≥126 mg/dl, a 2-h plasma glucose level of ≥200 mg/dl, or treatment with hypoglycemic agents. Dyslipidemia was defined as a low-density lipoprotein cholesterol level of ≥140 mg/dl, high-density lipoprotein cholesterol level of <40 mg/dl, triglyceride level of ≥150 mg/dl, or treatment with specific lipid-lowering agents. The baseline height and weight were recorded, and the body mass index (BMI) was calculated. Serum creatinine levels were measured using an enzymatic method. The estimated glomerular filtration rate (eGFR) was calculated using the Japanese Society of Nephrology formula [28] as follows:

eGFR (mL/min per 1.73 m2 =194 × serum creatinine 1.094 × age 0.287 (× 0.739 if female).

Randomization and allocation to treatment groups

Allocation to treatment arms was achieved using sealed opaque envelopes prepared by an independent assistant and consisted of random permuted blocks of size 8 with stratification according to proteinuria (1 g/gCr) and duration from the onset of urinary abnormality (3 years), as both could potentially affect the remission rate [17]. A sample size of 80 patients was calculated based on a significant level (α error) of 0.05, a β error of 0.2, a power (1−β) of 0.8 with predicted clinical remission rates of 40% and 70% in the control and ARB arms, respectively, at 12 months and a predicted dropout rate of 30% based on our historical population.

Study protocol

As shown in Fig. 1, control patients (n = 37) were scheduled to receive standard treatment with steroid pulse followed by oral prednisolone for 6 months and tonsillectomy within 6 months after steroid pulse therapy. Since steroid pulse are generally superior for rapid resolution of inflammatory process, patients with diffuse active lesion are supposed to be better for starting treatment with steroid pulse. Thus steroid pulse was performed prior to tonsillectomy in standard treatment. Patients in the ARB arm (n = 40) were scheduled to receive standard regimen plus candesartan for 6 months. Those who did not achieve remission in either arm, as determined by the presence of proteinuria at 12 months, were scheduled to receive candesartan which was planned to be titrated until the 24-month visit. High-dose methylprednisolone (0.5 g/day, three times a week for 3 consecutive weeks) as steroid pulse therapy was followed by oral prednisolone at an initial dose of 30 mg every other day with gradual tapering over six months. Tonsillectomy was performed after steroid pulse therapy during the first six months by an otolaryngologist after obtaining informed consent, regardless of the gross appearance of tonsils and even in the absence of episodes of recurrent tonsillitis or gross hematuria with tonsillitis. Candesartan was initially adminstered at a dose of 2–8 mg/day, followed

by titration to achieve remission of proteinuria to a max dose of 12 mg/day, unless symptomatic hypotension emerged as a complication.

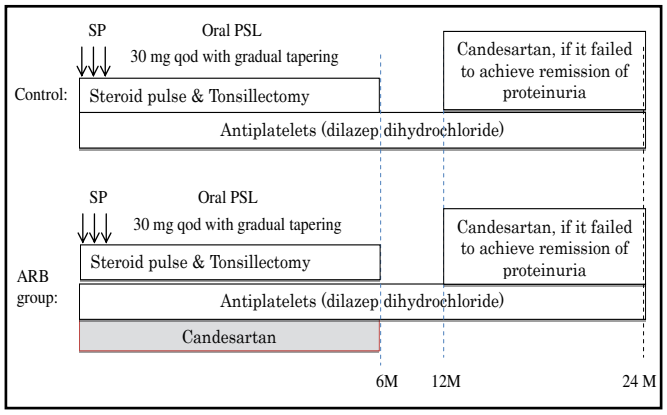

Fig. 1. Study protocol. ARB: angiotensin receptor blocker; M: months; PSL: prednisolone; SP: steroid pulse.

Outcome measures

The primary endpoint was the rate of clinical remission at 12 months, and the secondary endpoints were clinical remission rates at 6 and 12 months and the levels of urinary protein, urinary red blood cell, and estimated glomerular filtration rate. Previous study demonstrated that even trace proteinuria would be associated with renal event [29]. Thus, clinical remission was originally defined as a urinary protein level <0.2g/gCr and urinary red blood cell count <5/HPF lasting for a duration of 2–3 months, determined at two consecutive visits. However, Japanese society of nephrology proposed the criteria of clinical remission of IgA nephropathy, recently. Thus we determined remission rate using modified definition of urinary protein levels <0.3g/gCr according to the proposed definition [30]. We also examined the rate of clinical remission by subgroup of urinary protein level ≥ 1.0 g/gCr.

Measurement of urinary angiotensinogen

Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the kidneys and degrades angiotensin (Ang) II to Ang-(1–7); it is suggested to have an important role in the progression of hypertension and renal diseases, including IgA nephrolathy [31]. Thus, urinary Ang-(1-7) is a potential marker of intrarenal RAS. Contrarily, urinary angiotensinogen (AGT) is known to be a superior biomarker of intrarenal RAS[24]. Moreover, previous reports showed that urinary AGT levels were elevated in patients with IgA nephropathy, which positively correlated with renal angiotensinogen gene expression and Ang II immunoreactivity [21]. Additionally, its levels were reduced, accompanied with reduction of proteinuria after treatment with ARB[21]. Therefore, we measured urinary AGT levels as a marker of intrarenal RAS. Levels of urinary angiotensinogen (AGT) in spot urine to evaluate renal RAS were measured using a novel sandwich enzyme-linked immunosorbent assay at the University of Kagawa in Japan, as described previously [32, 33], and urinary AGT/creatinine ratio (UAGTCR, μg/gCr) was determined. The intra- and inter-assay coefficients of AGT measurements were <10%.

Histological analysis

Histological analysis was performed according to the Oxford classification of IgA nephropathy [33] by the same physician at the University Hospital of Ryukyus who was blinded to clinical information. The presence of extracapillary lesions was also assessed.

Statistical analyses

Baseline characteristics were expressed as mean (standard deviation [SD]), median (interquartile range) or N (%) and compared between randomized groups using Wilcoxon rank-sum test or chi-square test as appropriate. Effects of randomized treatment on the primary outcome (remission rate) at each time point was evaluated using univariable and multivariable logistic regression models. Stratified analysis by proteinuria (<1g/gCr vs ≥1g/gCr) was also conducted for the primary outcome. Outcomes of continuous variables (urinary protein, angiotensinogen and eGFR) at each time point were compared between randomized groups using one-way analysis of variance (ANOVA) for crude analysis and analysis of covariance (ANCOVA) for multivariable analysis. Urinary red blood cell grade between randomized groups were compared using multinomial logistic regression models. *P* values <0.05 were considered statistically significant. All

statistical analyses were performed in blinded fashion by Dr. Arima who did not recruit any patients using SAS release 9.4 (SAS Institute Inc, Cary, NC).

Results

Enrollment began on April 17, 2007 and ended on December 31, 2011; the last patient enrolled in the study ended treatment on April 27, 2014. A total of 77 eligible patients who provided written informed consent were randomly allocated to either the control (n = 37) or the ARB (n = 40) arm. Three patients in each group were lost to follow-up because they had not visited the clinic until 24M. Therefore, 34 patients in the control arm and 37 patients in the ARB arm completed the study with the final follow-up at 24 months (Fig. 2). The analysis was done by original assigned group including five patients who were lost of follow-up from 12M to 24M since their data for primary outcome were available.

Baseline clinical characteristics

As shown in Table 1, there were no significant differences in baseline clinical and histological characteristics including age, systolic blood pressure, eGFR, and urinary protein levels between the control and the ARB arms. The percentages of patients with urine protein ≥1g/gCr was 46% and 50% in the control and ARB arms, respectively.

Remission rates

Although, clinical remission defined as UP<0.3g/gCr and urinary red blood cell count <5/HPF) were comparable between the two treatment arms at 12 months, as well as at 6 and 24 months, remission rate at 24 month was

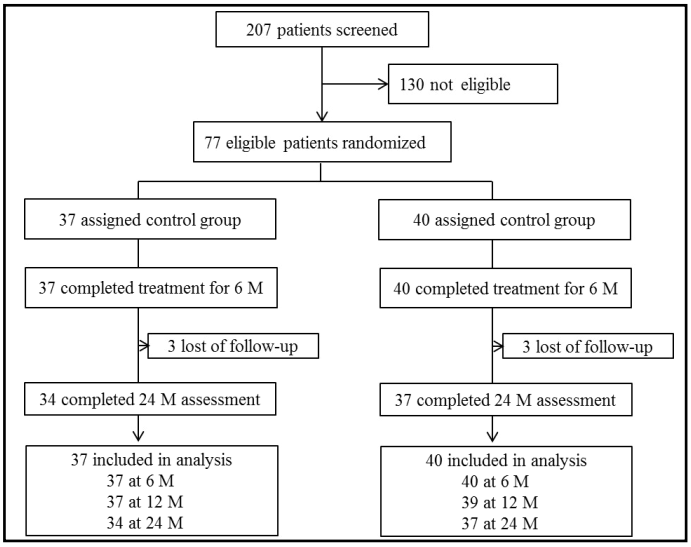

Fig. 2. Patient flow. Abbreviations are shown in the legend of Fig. 1.

Table 1. Baseline characteristics of patients after randomization. Data are expressed as means (standard deviation), medians (interquartile range), or numbers (%). ARB: angiotensin receptor blocker; ACE: angiotensin-converting enzyme; eGFR: estimated glomerular filtration rate; HPF: high-power field; MR: mineralocorticoid receptor; RAS: renin-angiotensin inhibitor; RBC: red blood cell

Characteristics	Control (N=37)	ARB (N=40)	P value
Age, years	35.8 (14.6)	36.3 (12.8)	0.863
Female	24 (65%)	19 (48%)	0.125
Years from onset, year	4 (2 to 8)	3 (1 to 7)	0.263
Body mass index (BMI), kg/m²	24.7 (4.0)	23.1 (3.1)	0.666
BMI ≥25kg/m²	13 (35%)	10 (25%)	0.332
Diabetes	0 (0%)	3 (8%)	0.089
Hypertension	7 (19%)	14 (35%)	0.113
SBP (mmHg)	120 (14)	121 (16)	0.764
DBP(mmHg)	73 (10)	73 (14)	0.959
Serum creatinine (mg/dl)	0.8 (0.2)	0.8 (0.2)	0.648
eGFR (ml/min/1.73m²)	85 (29)	84 (24)	0.988
Urinary protein (g/gCr)	0.9 (0.7 to 1.2)	1.0 (0.6 to 1.5)	0.968
Urinary protein >1g/gCr	17 (46%)	20 (50%)	0.722
Urinary sediment RBC count			
Grade0 (<5/HPF)	0 (0%)	5 (13%)	0.186
Grade1 (5-9/HPF)	7 (19%)	5 (13%)	
Grade2 (10-19/HPF)	9 (24%)	11 (28%)	
Grade3 (20-29/HPF)	8 (22%)	5 (13%)	
Grade4 (>30/HPF)	13 (35%)	13 (33%)	
Antihypertensive drugs	10 (27%)	15 (38%)	0.327
ARB	5 (14%)	7 (18%)	0.596
ACE inhibitors	2 (6%)	0 (0%)	0.131
RASI	6 (16%)	7 (18%)	0.883
Calcium channel blocker	6 (3%)	9 (23%)	0.487
Beta blocker	1 (3%)	1 (3%)	0.955
Alpha blocker	1 (3%)	0 (0%)	0.296
Diuretics	1 (3%)	2 (5%)	0.603
MR antagonists	0 (0%)	84 (24)	0.603
Pathologic parameters			
M1	21 (57%)	15 (38%)	0.091
En1	13 (35%)	18 (45%)	0.378
S1	24 (65%)	28 (70%)	0.631
Ex1 (%)	11 (6-22)	13 (8-24)	0.313
Tubular atrophy/ Interstitial fibrosis			
T0	25 (68%)	27 (69%)	0.178
T1	9 (24%)	12 (31%)	
T2	3 (8%)	0 (0%)	

rather favor for control arms as shown in Table2. Titration of candesartan from 12-months improved remission rate at 24-monthsin either arms. The dose of candesartane (median, minimum to max) were 8 mg (2mg–12mg) and 8mg (2–12mg), respectively.

Remission rates among patients with urinary protein levels <1 g/gCr were 45% and 35% at 6 months, 50% and 52.6% at 12 months, and 77.8% and 57.9% at 24 months in the control and ARB arms, respectively. Among patients with urinary protein levels ≥1 g/gCr, remission rates were 29.4% and 35.0% at 6 months, 47.1% and 25% at 12 months, and 64.7% and 45% in the control and ARB arms, respectively (Table 3).

Table 2. Remission rates at each visit. ARB: angiotensin receptor blocker * Adjusted for age, sex, obesity, hypertension, diabetes mellitus, estimated glomerular filtration rate at baseline and randomized group (model 1). **model1 + baseline angiotensinogen. ***model1 + angiotensinogen change (from baseline to 6 months)

Time	Control (N=37)	ARB (N=40)	p value	P adjusted *	P adjusted **	P adjusted ***
6 months	14/37 (37.8%)	14/40 (35.0%)	0.800	0.494	0.751	0.808
12 months	18/37 (48.7%)	15/39 (38.5%)	0.371	0.666	0.799	0.770
24 months	25/35 (71.4%)	20/39 (51.3%)	0.076	0.055	0.145	0.164

Table 3. Remission rates at each visit stratified by baseline urinary protein level. Remission was defined as <0.3g /gCr and <5 red blood cell casts/high-power field. ARB: angiotensin receptor blocker

Time	Basal urinary protein	Control (N=37)	ARB (N=40)
6 months	<1 g/gCr	9/20 (45.0%)	7/20 (35.0%)
	≥1 g/gCr	5/17 (29.4%)	7/20 (35.0%)
12 months	<1 g/gCr	10/20 (50.0%)	10/19 (52.6%)
	≥1 g/gCr	8/17 (47.1%)	5/20 (25.0%)
24 months	<1 g/gCr	14/18 (77.8%)	11/19 (57.9%)
	≥1 g/gCr	11/17 (64.7%)	9/20 (45.0%)

Urinary protein level, red blood cell grade, and estimated glomerular filtration rate

Mean urinary protein levels of 1.02 and 0.97 g/gCr in the control and ARB arms, respectively, at baseline were markedly reduced to 0.21 and 0.11 g/gCr at 6 months (Fig. 3). Although mean urinary protein in the ARB arm was significantly lower than that in the control arm at 6 months, mean urinary protein levels were comparably maintained at around 0.1g/gCr afterword.

Assessment of hematuria revealed that urinary red blood cell (RBC) grades were significantly lower in the control arm than in the ARB arm at all follow-up points (Table 4). No significant changes in eGFR were observed in either treatment arm at 24 months (Fig. 4).

Blood pressure

Blood pressure was well controlled in both groups at baseline. Mean systolic and diastolic blood pressure values (SD) in the control and ARB arms for 6, 12, and 24 months are indicated in Table 5. Systolic blood pressure was strictly and comparably controlled at around 110 mmHg in both groups from 6 months until 24 months.

Urinary angiotensinogen levels

As shown in Fig. 5, urinary AGT levels were reduced at 6 months and remained at comparably low levels thereafter in both the control and ARB arms. Mean urinary AGT in the ARB arm was transiently increased at 12 months after discontinuation of ARB. Conversely, urinary AGT levels in control arm were sustainably decreased at 12 months compared to 6 months. Importantly, mean urinary AGT levels in both treatment arms were decreased at 24 months after the addition of ARB to treatment of patients who not achieve remission based on the presence of proteinuria.

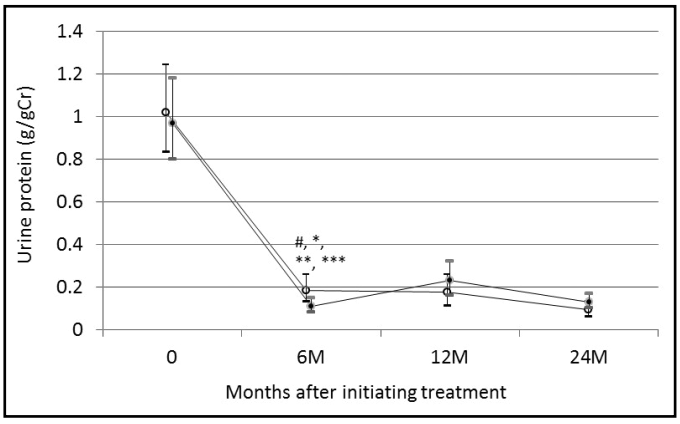

Fig. 3. Time course of urinary protein by randomized group. Blank circles represent average in the control group; black squares, average in the ARB group; vertical lines, confidence intervals. # p=0.002 in crude analysis. * p=0.005 after adjustment for age, sex, obesity, hypertension, diabetes mellitus, estimated glomerular filtration rate at baseline and randomized group (model 1). **model1 + baseline angiotensinogen, p=0.0004. ***model1 + angiotensinogen change (from baseline to 6 months), p=0.001.

Table 4. Time course of urinary red blood cell scores over time by randomized group. P values were tested using chi-squared test. RBC: red blood cell; ARB: angiotensin receptor inhibitor. *model1: Adjusted for age, sex, obesity, hypertension, diabetes mellitus, estimated glomerular filtration rate at baseline and randomized group. **model1 + baseline angiotensinogen. ***model1 + angiotensinogen change (from baseline to 6 months)

Time	U RBC	Control	ARB	P value	P adjusted *	P adjusted **	P adjusted ***
Baseline	0	0/37 (0%)	5/39 (13%)	0.186	0.371	0.684	0.207
	1	7/37 (19%)	5/39 (13%)				
	2	9/37 (24%)	11/39 (28%)				
	3	8/37 (22%)	5/39 (13%)				
	4	13/37 (35%)	13/39 (33%)				
6 months	0	21/37 (57%)	15/40 (38%)	0.251	0.064	0.156	0.042
	1	7/37 (19%)	13/40 (33%)				
	2	6/37 (16%)	4/40 (10%)				
	3	1/37 (3%)	3/40 (8%)				
	4	2/37 (5%)	5/40 (13%)				
12 months	0	30/37 (81%)	21/37 (57%)	0.060	0.005	0.013	0.021
	1	6/37 (16%)	11/37 (30%)				
	2	1/37 (3%)	0/37 (0%)				
	3	0/37 (0%)	2/37 (5%)				
	4	0/37 (0%)	3/37 (8%)				
24 months	0	29/34 (85%)	23/36 (64%)	0.008	0.028	0.032	0.014
	1	3/34 (9%)	2/36 (6%)				
	2	0/34 (0%)	7/36 (19%)				
	3	2/34 (6%)	0/36 (0%)				
	4	0/34 (0%)	4/36 (11%)				

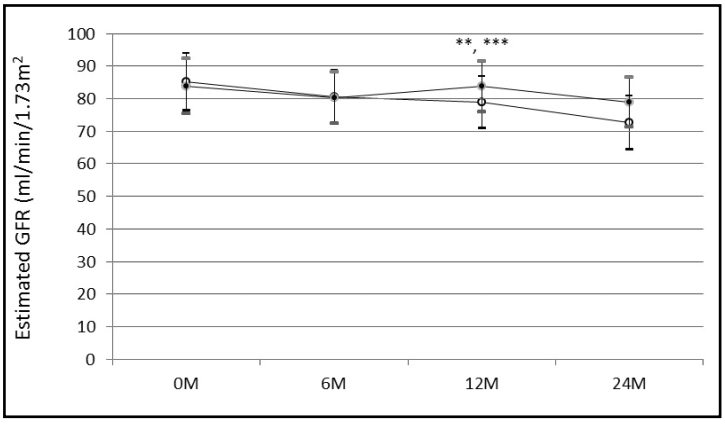

Fig. 4. Time course in estimated glomerular filtration rate by randomized group. Blank circles represent average in the control group; black squares, average in the ARB group; vertical lines, confidence intervals. Adjustment for age, sex, obesity, hypertension, diabetes mellitus, estimated glomerular filtration rate at baseline and randomized group (model 1). *model1 + baseline angiotensinogen, p=0.032. **model1 + angiotensinogen change (from baseline to 6 months), p=0.024.

Table 5. Time course of blood pressure. *Wilcoxon test

Variable	Time	Control	ARB	P value*
Systolic blood pressure, mmHg	Baseline	120 (14)	121 (16)	0.764
	6 months	112 (12)	109 (14)	0.087
	12 months	113 (12)	113 (11)	0.996
	24 months	113 (12)	112 (13)	0.581
Diastolic blood pressure, mmHg	Baseline	73 (10)	73 (14)	0.959
	6 months	69 (9)	67 (9)	0.268
	12 months	70 (8)	70 (9)	0.836
	24 months	68 (11)	68 (10)	0.897

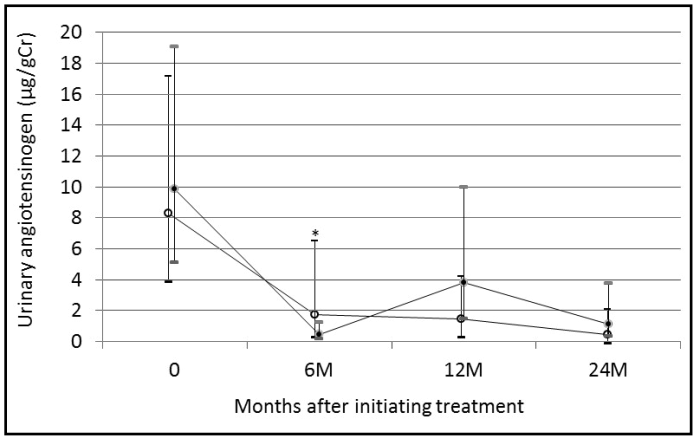

Fig. 5. Time course of urinary anginotensinogen by randomized group. Blank circles represent average in the control group; black squares, average in the ARB group; vertical lines, confidence intervals. *p=0.031 after adjustment for age, sex, obesity, hypertension, diabetes mellitus, estimated glomerular filtration rate at baseline, randomized group and baseline angiotensinogen. AGT was log-transformed to remove skewness. Mean values were obtained by back-transformation. P values were tested using analysis of variance.

Adverse events

All participants completed 6 months of therapy without any serious adverse events such as infections requiring hospitalization. One patient in the control arm and two patients in the ARB arm experienced worsening of hemoglobin A1c, which was around 6% at 6 months; the patient in the control arm was treated with an alpha-glucosidase inhibitor and glucose controls of the two patients in the ARB arm naturally improved with steroid dose reduction. One patient in the control arm temporarily developed partial alopecia. One female patient in each of the ARB and control arms became pregnant at 6 and 23 months after the commencement of the study, and both discontinued all medications including study drugs.

Discussion

The findings of this trial demonstrated that the significant reduction in proteinuria observed in patients receiving TSP-based therapy without candesartan at 6 months was further enhanced by the concomitant use of candesartan in patients with active IgA nephropathy and active glomerular lesions. However, concomitant use of candesartan independent of its blood pressure-lowering effects did not improve the clinical remission rate achieved by TSP-based treatment at neither 6 nor 12 months. Importantly, candesartan administration to patients who did not achieve clinical remission in either treatment arm, regardless of their blood pressure levels, effectively reduced urinary protein levels and eventually led to increased clinical remission rates.

Previous studies suggested that the interplay between inflammation and RAS might play a role in the clinical progression of IgA nephropathy [34, 35]. Moreover, treatment with ARBs regardless of the blood pressure levels was demonstrated to be effective in reducing

proteinuria [36]. However, short-term, concomitant use of candesartan was not associated with improvement in the clinical remission rate among IgA nephropathy patients treated with TSP in the present study. Notably, while candesartan was administered only in the ARB arm during the first 6 months, the urinary protein levels of most patients in both treatment arms were around 0.2 g/gCr within 6 months after the study commencement date and were maintained at comparable levels until 12 months. These findings hint at the favorable impact of the TSP-based therapy itself on the resolution of active glomerulonephritis, as demonstrated by previous studies [7, 37]. Therefore, inhibition of RAS with ARBs such as candesartan might have a limited role in achieving remission during the early phase of TSP-based treatment. Alternatively, resolution of inflammatory processes by blocking angiotensin type 1 receptor might be limited, since angiotensin II was shown to activate nuclear transcription factor-kappa B (NF-κB) via angiotensin type 2 receptor as well as type 1 receptor [25]. In general, the effects of RAS inhibitors are supposed to be primarily mediated by the alteration of glomerular hemodynamics. In reality, addition of candesartan for the first 6 months significantly reduced proteinuria to 0.1 g/g Cr with greater reduction of the urinary AGT level. Therefore, the duration of treatment with candesartan appeared to sufficiently reduce proteinuria in the early phases of TSP-based treatment. Moreover, RAS blockade with candesartan was supposed to be sufficient to suppress intrarenal RAS and proteinuria, although we did not use dual blockade with ARB and ACE inhibitors.

Although an additional benefit with candesartan during the early phase of TSP-based therapy in IgA nephropathy was not demonstrated, its addition to therapy regardless of the blood pressure levels of patients was beneficial in reducing proteinuria and eventually improving the clinical remission rate in patients who would not otherwise achieve clinical remission during the later phase of TSP-based therapy. Moreover, reducing proteinuria by addition of candesartan was accompanied by a reduction in urinary AGT. Since renal RAS was suggested to regulate glomerular hemodynamics [38], these observations suggested that glomerular hypertension might be responsible for residual proteinuria. Alterations in the autoregulation system in afferent arterioles, which maintains glomerular blood pressure levels at around 50 mmHg over a wide range of systemic blood pressure levels, leads to the direct transmission of systemic blood pressure to glomeruli without sufficient reduction. Therefore, even a normal range of systemic blood pressure might be associated with glomerular hypertension and might result in increased proteinuria. We recently reported that hyalinosis in renal arterioles, a potential marker for dysregulation of the autoregulation system, was associated with higher urinary protein levels and greater reductions in eGFR than those seen in patients without hyalinosis, even within the normal range of blood pressure in IgA nephropathy [39]. Moreover, we reported that uric acid may augment susceptibility for hypertensive glomerular damage in association with evoking renal arteriolopathy in IgA nephropathy [40]. Consistent with this hypothesis, uric acid was reportedly associated with the progression of IgA nephropathy [41]. Titration of ARB regardless of the systemic blood pressure levels of patients might be crucial for achieving clinical remission, especially in patients who have not yet achieved it.

Surprisingly, this study showed that even TSP-only treatment successfully reduced the urinary levels of AGT, a marker of renal RAS status. Previous studies demonstrated that activation of renal RAS observed in patients with IgA nephropathy could be reversed by ARBs [21]. The benefit of TSP in IgA nephropathy appears to be based on its efficacy in resolution of inflammation in the glomerulus and reduction in the production of abnormally glycosylated IgA in the tonsils [42]. NF-κB, a mediator of inflammation, is proposed to be involved in the development of IgA nephropathy [43]. Since NF-κB is a transcriptional activator of AGT [26] that modulates angiotensin II-mediated responses, resolution of inflammation by TSP alone might be sufficient even in the absence of ARBs. Moreover, TSP-mediated reduction in proteinuria might underlie the decreases observed in urinary AGT levels, since proteinuria was reported to be associated with NF-κB-dependent activation of renal RAS [44]. Alternatively, increases in urinary AGT might result from injured glomerular

capillary and might return to normal levels following resolution of glomerular inflammation. Additional treatment with candesartan effectively reduced both proteinuria and urinary AGT in patients not achieving clinical remission in both treatment arms, suggesting that alterations in renal AGT might be contributing to residual proteinuria in these patients, which could be reversed by ARBs, in agreement with a previous report [21].

The present study has several limitations. First, the number of enrolled subjects was relatively low, which might adversely affect the robust elucidation of the efficacy of short-term candesartan treatment. Given that even TSP-only treatment led to significant reductions in urinary protein demonstrating the additional benefit of candesartan for remission might be challenging. Second, the duration of follow-up oral steroid therapy for 6 months after TSP might be short, given that treatment with oral prednisolone after TSP was maintained for 12 months in the original protocol reported by Hotta *et al.* which showed more favorable remission rates [7]. Third, the grade of hematuria was relatively higher in the ARB arm at 6 months, suggesting the presence of potential bias in disease activity between the two treatment arms that was not evident by usual clinical and histological assessment conducted at baseline. Fourth, since relatively mild cases were recruited in this study, the findings might not be applicable to more severe IgA nephropathy cases. Finally, urinary findings could not be followed up beyond 24 months after study initiation; thus, whether clinical remission might be maintained long term and result in favorable renal outcomes remain to be determined.

Conclusion

In the patients with active IgA nephropathy, the clear benefit of concomitant use of the ARB candesartan with TSP in achieving clinical remission during early-phase of treatment, independent of its blood pressure-regulatory function, could not be demonstrated. TSP alone could suppress intrarenal RAS in the absence of RAS inhibition; moreover, about 50% of the patients achieved clinical remission, suggesting a primary role for inflammation in IgA nephropathy. In cases where clinical remission might not be achieved by TSP treatment, addition of an ARB such as candesartan, with titration as guided by residual urinary protein but not by blood pressure level, might be beneficial for achieving clinical remission. Further investigation is necessary to elucidate the role of TSP, RAS inhibition, and the combination of these distinct approaches in clinical remission of IgA nephropathy.

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