

Angiotensin-Converting Enzyme Inhibitor versus Angiotensin 2 Receptor Antagonist Therapy and the Influence of Angiotensin-Converting Enzyme Gene Polymorphism in IgA Nephritis

Keng-Thye Woo,¹FRACP, M Med (Int Med), FAMS, Yeow-Kok Lau,¹PhD, Choong-Meng Chan,¹MBBS (Newcastle), MRCP (UK), FAMS, Kok-Seng Wong,¹MBBS, M Med (Int Med), FAMS

Abstract

Introduction: In this study of 109 patients with IgA nephritis (IgAN), we compared the long-term effects on patients treated with angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor antagonist (ATRA) alone with respect to renal outcome in terms of ESRF from 1995 to 2006. The renal outcome is also correlated with the ACE gene ID polymorphism to study its influence on response to ACEI/ATRA therapy. **Materials and Methods:** Seventy-seven patients were on treatment with ACEI/ATRA (22 on ACEI alone, 47 on ATRA alone and 8 on both). The other 32 patients were on no treatment (control group). **Results:** Compared to controls, treated patients had lower serum creatinine ($P < 0.001$), lower proteinuria ($P < 0.001$) and fewer number progressing to ESRF ($P < 0.001$). For those with the II and ID genotype there were significantly fewer patients with ESRF in the treatment group. With the DD genotype, treatment did not change the poor renal outcome with regard to ESRF. Patients on ACEI therapy had a higher incidence of ESRF compared to those on ATRA ($P < 0.001$). For the control group, the projected number of years-to-ESRF was 10 years. For those on ACEI therapy it was 11 years, and for those on ATRA therapy it was 24 years. Among patients with the II genotype, those treated with ATRA had significantly less incidence of ESRF compared to those treated with ACEI ($P < 0.001$). **Conclusion:** ATRA therapy was found to be effective in retarding disease progression to ESRF in IgAN compared to ACEI therapy. Genotyping showed better response to ATRA therapy only for those with the II genotype.

Ann Acad Med Singapore 2008;37:372-6

Key words: End-stage renal failure, Genotyping, Reciprocal creatinine plots

Introduction

Angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor antagonists (ATRA) are both well established drugs utilised to help retard the progression of chronic kidney diseases to end-stage renal failure (ESRF), either by reducing proteinuria or even reversing mild renal impairment and restoring normal renal function in some cases.^{1,2} Recently Suissa et al³ reported that the use of ACEI in diabetic patients is not associated with a long-term decreased risk of ESRF. It has been suggested that while ACEI could provide an early benefit to the kidney, it could cause damage to the kidney in the long term.⁴

In an earlier publication,⁵ where we reported that ACEI/ATRA therapy decreases proteinuria by improving glomerular permselectivity in IgA nephritis (IgAN), we found ATRA to be superior. Our data showed that the majority of the non-responders were on ACEI compared to the responders who were on ATRA, suggesting that ATRA is more effective in decreasing proteinuria. Hence we were

not surprised when Suissa et al³ reported that ACEI was not protective of ESRF.

This present report was based on a retrospective analysis of 109 patients with IgAN to study the long-term effects of being treated with ACEI alone or ATRA alone with respect to renal outcome in terms of development of ESRF over 12 years. This paper also attempted to correlate the renal outcome with the ACE gene ID polymorphism as we believe that the ACE gene profile of the patients may influence response to ACEI/ATRA therapy.

Materials and Methods

Patients

From a renal biopsy database of 176 patients with IgA Nx, we found that 151 were still on follow-up at the Renal Clinic. We managed to obtain written consent from 109 Chinese patients to study their ACE gene polymorphism. This was a retrospective study with access to medical records from 1995 to 2006 (12 years) to assess the effects

¹ Department of Renal Medicine, Singapore General Hospital, Singapore
Address for Correspondence: Prof Woo Keng-Thye, Senior Consultant, Department of Renal Medicine, Singapore General Hospital, Outram Road, Singapore 169608. Email: woo.keng.thye@sgh.com.sg

of ACEI and ATRA therapy (Treatment group) compared to those not on either therapy (Control group). The ACE gene ID genotype was also studied in order to compare the effect of ID polymorphism on the response to ACEI/ATRA therapy. There were no significant differences in the various parameters between the treatment and control group on recruitment into the study (Table 1). In the control group, for treatment of hypertension, patients were on atenolol, propranolol, hydralazine and amlodipine. Seventy-seven patients were on treatment with ACEI/ATRA (22 on ACEI alone, 47 on ATRA alone and 8 on both). The mean dosage of ACEI (Enalapril) was 19 ± 14 mg/day and ATRA (Losartan) 95 ± 16 mg/day, final dose in both groups.

For this study, each patient was sampled for 2 mL EDTA blood for DNA extraction and ACE gene ID genotyping. All the patients gave their informed consent to participate in the study after the nature of the study was explained to them. The study was approved by the local institution review board and the hospital ethics committee.

Table 1. Comparing Clinical Profiles of Patients with Controls

	Treat n = 77	Control n = 32	P value
Sex (F:M)	42:35	12:20	ns
Age at biopsy (y)	33 ± 10	30 ± 11	ns
Hypertension (Yes:No)	35 : 42	13 : 19	ns
Follow-up duration (y)	12.0 ± 6.4	9.5 ± 5.8	= 0.05
Serum creatinine (mg/dL)			
Before	1.4 ± 0.4	1.5 ± 0.4	ns
After	2.6 ± 2.1	5.1 ± 2.9	<0.001
Urinary total protein (g/day)			
Before	2.0 ± 0.7	2.2 ± 1.7	ns
After	1.0 ± 0.8	1.8 ± 1.0	<0.001
Blood pressure (mm Hg)			
Systolic before	136 ± 12	133 ± 13	ns
Diastolic before	83 ± 8	86 ± 6	<0.05
Systolic after	132 ± 11	129 ± 12	ns
Diastolic after	82 ± 8	83 ± 7	ns
Outcome			
Non-ESRF	64	7	<0.001
ESRF	13	25	($\chi^2 = 37.3$)
ACE genotype			
II	34	16	ns
ID	33	11	
DD	10	5	

ACE: angiotensin converting enzyme; ESRF: end-stage renal failure; ns: not significant

Determination of ACE Insertion/Deletion Genotypes

DNA was extracted from 0.2 mL EDTA-blood using the QIAamp DNA blood extraction kit (QIAGEN, Germany). Genotyping was done using the method of Vleming et al.⁶ The 50 µL of reaction mixture consisted of 50 ng DNA, 1X PCR buffer (Fermentas), primers concentration 0.4 µM (forward 5'-CTGGAGACCACTCCCATCCTTTCT-3'; reverse 5'-GATGTGGCCATCACATTCGTCAGAT-3'), 0.2 mM dNTPs and 1 unit Taq polymerase (Fermentas). Amplification was carried out in an automated thermocycler (GeneAmp 9700, USA) for 35 cycles (94°C, 30 s; 60°C, 45 s and 72°C, 60 s). Products were separated in 2% agarose gel and visualised by ethidium bromide staining. Amplification of the I allele produced 1 band at 490 base pair (bp) for homozygote II. Amplification of the D allele produced 1 band at 190 bp for homozygote DD. Both bands at 490 bp and 190 bp were produced by heterozygote. Mistyping ACE heterozygotes as DD homozygotes had been reported. Therefore, all DD cases were subject to confirmation with a second PCR, performed using the insert-specific forward primer 5'-TTTGAGACGGAGTCTCGCTC-3' together with the same reverse primer above.⁶ A true DD genotype would product at the 409-bp band, whereas ID and II genotypes should.

Statistics

SPSS 10.0 for Windows was used to calculate Pearson's chi-square for comparing categorical data and Student's *t*-test for evaluating significance of difference between means of numeric data. Years-to-ESRF was estimated for each patients from reciprocal creatinine plot⁷ and extrapolation to the reciprocal of 8 mg/dL serum creatinine, our defined ESRF point. Patients who had improved serum creatinine values had regression lines with positive slopes and no extrapolated estimates of years-to-ESRF. Therefore to enable significance analysis to proceed, the value of 30 years-to-ESRF was given to these cases as the calculated estimated value for 32 controls was 10 ± 11 years (mean ± SD).

Results

Table 1 compares data between the 77 patients in the treatment group and the 32 patients in the control group at the entry and end-points of the study. At entry, there were no significant differences between the treatment and control groups in the various parameters except for a longer duration of follow-up in the treatment group (12 years versus 9.5 years, $P = 0.05$). But at end-point, the mean serum creatinine in the control group was worse than the treatment group ($P < 0.001$). Proteinuria was also worse in the control group compared to the treatment group at end-point ($P < 0.001$). With regard to renal outcome, there were 25 patients with ESRF in the control group compared to 13

Table 2. Impact of ACE ID Genotype on Treatment and Disease Progression

	End-stage	Normal/Impaired	P value
Genotype II			
Control	13	3	<0.001
Treated	5	29	
Genotype ID			
Control	9	2	<0.001
Treated	5	28	
Genotype DD			
Control	3	2	ns
Treated	3	7	
All patients			
Control	25	7	<0.001
Treated	13	64	

in the treatment group ($\chi^2=37.3, P<0.001$). Thus treatment does reduce the number of patients progressing to ESRF.

Table 2 is a grouping of the patients in the treatment and control group according to their genotype profile. For those with II and ID genotype there were significantly fewer patients with ESRF in the treatment group ($P<0.001$). For those with the DD genotype, there was no significant difference in renal outcome. With the DD genotype, treatment did not change the poor renal outcome with regard to ESRF.

Table 3 shows the impact of the drug on treatment and disease progression. Patients on ACEI therapy had a higher incidence of ESRF compared to those on ATRA (9 versus 2 patients, $\chi^2=15, P<0.001$). There was also a higher incidence of patients with ESRF and renal impairment in the ACEI-treated group compared to those on ATRA therapy ($\chi^2=12.4, P<0.001$).

Table 4 compares the various parameters between patients treated with ACEI and those treated with ATRA. There was no significant differences between the various parameters at entry but at end-point, the serum creatinine and the urinary protein were significantly higher in the ACEI-treated patients compared to those treated with ATRA ($P<0.001$ and $P<0.005$ respectively). There were also more patients who developed ESRF in the ACEI-treated group, compared to those in the ATRA treated group ($P<0.001$).

Table 5 shows the impact of ACE ID genotype on those treated with ACEI and ATRA. For those patients with the II genotype, those treated with ATRA had significantly less incidence of ESRF compared to those treated with ACEI ($\chi^2=14.6, P<0.001$). There was no such difference for patients with the ID and the DD genotype.

Table 3. Impact of Drug on Treatment and Disease Progression

	End-stage	Normal/Impaired	P value
Drug			
ACEI	9	13	<0.001
ATRA	2	45	($\chi^2=15.0$)
	End-stage/Impaired	Normal	P value
Drug			
ACEI	16	6	<0.001
ATRA	13	34	($\chi^2=12.4$)

ACEI: angiotensin converting enzyme inhibitors; ATRA: angiotensin II receptor antagonists

Table 4. Comparing Profiles of Patients with ACEI versus ATRA Treatment

	ACEI n = 22	ATRA n = 47	P values
Sex (F:M)	9:13	29:18	ns
Age at biopsy (y)	33 ± 9	33 ± 10	ns
Hypertension (Yes/No)	11:11	20:27	ns
Follow-up duration (y)	13.0 ± 6.4	11.0 ± 6.4	ns
Serum creatinine (mg/dL)			
Before	1.3 ± 0.3	1.4 ± 0.4	ns
After	4.4 ± 2.7	1.8 ± 1.3	<0.001
Urinary total protein (g/day)			
Before	2.0 ± 0.5	2.0 ± 0.7	ns
After	1.3 ± 0.7	0.8 ± 0.6	<0.005
Blood pressure (mmHg)			
Systolic before	140 ± 11	133 ± 11	<0.02
Diastolic before	84 ± 7	81 ± 86	ns
Systolic after	134 ± 12	130 ± 11	ns
Diastolic after	80 ± 8	81 ± 8	ns
Outcome			
Non-ESRF	13	45	<0.001
ESRF	9	2	
ACE genotype			
II	4	25	<0.02
ID	12	18	
DD	6	4	

ACE: angiotensin converting enzyme; ESRF: end-stage renal failure; ns: not significant

Table 6 and Figure 1 show the effects of therapy with projected years-to-ESRF estimated from reciprocal creatinine plots. For the control group, the projected number of years-to-ESRF (mean ± SD) was 10 ± 11 years. For those on ACEI therapy it was 11 ± 9 years, and for those on ATRA therapy it was 24 ± 12 years. There was a significant difference ($P<0.001$) in the number of years-to-ESRF

Table 5. Impact of ACE ID Genotype on ACEI versus ATRA Treatment

	End-stage	Normal/Impaired	P value
Genotype II			
ACEI	3	1	<0.001
ATRA	1	24	($\chi^2 = 14.6$)
Genotype ID			
ACEI	3	9	0.125
ATRA	1	17	($\chi^2 = 2.3$)
Genotype DD			
ACEI	3	3	0.091
ATRA	0	4	($\chi^2 = 2.9$)
All patients			
ACEI	9	13	<0.001
ATRA	2	45	($\chi^2 = 15.0$)

ACEI: angiotensin converting enzyme inhibitors; ATRA: angiotensin II receptor antagonists

Table 6. Effects of Treatment on Years-to-ESRF Estimates* (mean \pm SD)

	Years-to-ESRF	P value
Control (n = 32)	10 \pm 11	
vs ACEI (n = 22)	11 \pm 9	ns
vs ATRA (n = 47)	24 \pm 9	<0.001
vs ACEI/ATRA (n = 8)	24 \pm 12	<0.01
ACEI (n = 22)	11 \pm 9	
vs ATRA (n = 47)	24 \pm 9	<0.001
vs ACEI/ATRA (n = 8)	24 \pm 12	<0.005
ATRA (n = 47)	24 \pm 9	
vs ACEI/ATRA (n = 8)	24 \pm 12	ns

ACEI: angiotensin converting enzyme inhibitors; ATRA: angiotensin II receptor antagonists; ESRF: end-stage renal failure; ns: not significant

* Estimated from reciprocal creatinine plots and extrapolated to 8 mg/dL creatinine

between those on ACEI therapy (11 \pm 9 years) versus those on ATRA therapy (24 \pm 9 years).

Discussion

The data from this observational study, which compares the efficacy of ACEI and ATRA to prevent ESRF in patients with IgAN, show that the long-term renal outcome in terms of ESRF for those treated with ACEI is not very different from the control group. In contrast, patients treated with ATRA saw a doubling of the time to reach ESRF: 24 years for ATRA group compared to 11 years for the ACEI group. Even though this is not a prospective randomised clinical trial, the various parameters at entry for both the ACEI and the ATRA treated group were not different. The BP control in both groups at entry and end-point was the same. The dose of ACEI and ATRA were comparable in terms of equivalent clinical strength.

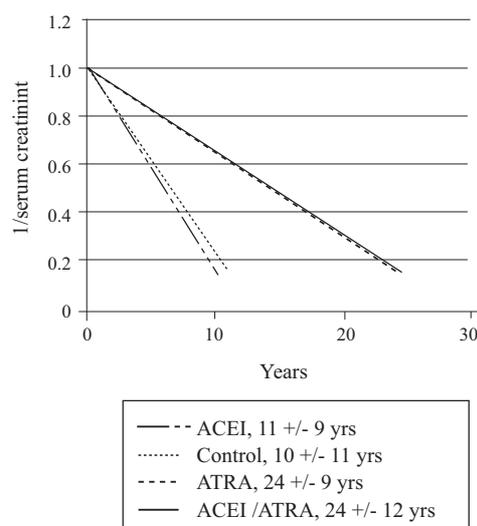


Fig. 1. The group means of reciprocal creatinine plots.

These data support the observation by Suissa et al³ in diabetic patients, where they reported that the use of ACEI in these patients was not associated with a long-term decreased risk of ESRF. This data are also consistent with our earlier observation on ACEI/ATRA therapy, where we reported that ATRA was superior in reducing proteinuria and more protective against ESRF compared to ACEI.⁸ Supporting the notion that ACEI can be deleterious, Hamming et al⁹ provided animal data to demonstrate that the long-term use of ACEI led to renal fibrosis resulting in renal damage to the kidney in the long term. Our data and those of Suissa et al³ are at best observational data and suggest the need for long-term randomised prospective control trials in patients on therapy with ACEI alone and ATRA alone. In our study, there were only 8 patients treated with ACEI and ATRA at the same time. With these 8 patients, years to ESRF estimates were similar to those treated with ATRA alone.

In this study, we also investigated the effects of ACE ID polymorphism and its influence on renal outcome and response to ACEI/ATRA therapy. Previously, we reported that patients with IgAN and the DD genotype were associated with ESRF.¹⁰ The present study again shows that the DD genotype is associated with a higher incidence of progression to ESRF. In contrast, the II genotype is renoprotective as fewer patients with the II genotype develop ESRF.

The ACE gene influences the renin activity of the renin-angiotensin system and those patients with the D allele, with the higher levels of ACE concentration would be expected to do poorly compared to those with the I allele where the ACE levels are lower.¹¹ Hence patients with the ID genotype would fall between the II and DD genotype in terms of renoprotection. This is borne out in our data shown in Table 2.

However, when we examined the effects of genomics on renal outcome, comparing those on ACEI alone with those on ATRA alone (Table 5), only those treated with ATRA with the II genotype were protected. This implies that even if a patient has the II genotype, if he is not on treatment or on treatment with only ACEI and not ATRA, the renoprotective effect is not apparent. Unfortunately, the number of patients on ACEI alone with the II genotype is very small (only 4) compared to 25 on ATRA alone with the II genotype. Statistically, a comparison with such small numbers would be flawed.

As a whole, comparing the patients in the treatment and those in the control group, our data do suggest that the II genotype is renoprotective as opposed to the DD genotype which is associated with ESRF. Two recent meta-analysis also support the finding that the SD genotype carries a worse renal outcome in IgAN.^{12,13}

Ng et al,¹⁴ in a meta-analysis of 14,724 diabetic patients, reported a similar protective role of the II genotype for Asian patients with type II diabetic nephropathy, whereby there was a reduction in the number with ESRF from 1994 to 2004 when they were treated with ACEI/ATRA. In contrast, those patients with the D allele had a deleterious outcome in terms of ESRF.

Seki et al,¹⁵ another group of Japanese workers, reported a similar renoprotective effect in 18 Asian patients with the II genotype with type II diabetes mellitus when treated with ACEI/ATRA, in contrast to those with the DD genotype.

It is believed that the differential drug responses could be explained by the ACE gene profile of the patients. So et al,¹⁶ studying 2089 Chinese patients with type II diabetes, reported similarly good renal outcome for those with the II genotype in contrast to those with the DD genotype.

In terms of pharmacogenomics, a person's genomic make-up may modify the target availability or function of a drug. The drug response is hence modified as a result. Arising from the interplay between genetic effects during drug therapy and the direct effects of drugs on gene transcription, individuals may respond differently, depending on their genomic make-up. This could explain the conflicting results between studies on Caucasians and Asians.

Like diabetic nephropathy, IgAN is a very common kidney disease worldwide. In both IgAN and diabetic nephropathy, the mesangial cell proliferation is a key step in the pathogenesis. So far the studies reporting a renoprotective effect of the II genotype in diabetics have been carried out on Asians. Our present data showing that IgAN patients with II genotype respond better to ACEI/ATRA therapy and are protected from ESRF are consistent with the findings in Asian patients with type II diabetic nephropathy. We postulate that in both diseases, the ACE gene may play a crucial role in the response of these

patients to ACEI/ATRA therapy. However a study from Korea by Han et al¹⁷ showed that renal preservation with ACEI was better in the DD than II genotype.

One of the main mechanisms could be the role of ACEI/ATRA in suppressing transforming growth factor-beta (TGFβ). TGFβ stimulates production of the extracellular matrix and induces glomerular sclerosis.¹⁵ Hashimoto et al¹⁸ suggests that ATRA is more useful than ACEI for patients with the allele I as it induces angiotensin type 2 receptor activation, which may reduce TGFβ concentrations.

In summary, ATRA therapy was found to be effective in retarding progression to ESRF in IgAN, but not ACEI. Treatment significantly reduced the incidence of ESRF only for patients with the genotype II and not those with the DD genotype.

REFERENCES

- Ruggenenti P, Perna A, Benini R, Bertani T, Zoccali C, Maggiore Q, et al; Investigators of the GISEN Group. In chronic nephropathies prolonged ACE inhibition can induce remission: dynamics of time-dependent changes in GFR. *J Am Soc Nephrol* 1999;10:997-1006.
- Shahinfar S, Dickson T, Zhang Z, Keane WF, Brenner BM; RENAAL Investigators. Baseline predictors of end-stage renal disease risk in patients with type 2 diabetes and nephropathy: new lessons from the RENAAL Study. *Kidney Int* 2005;93(Suppl):S48-S51.
- Suissa S, Hutchison T, Brophy JM, Kezouh A. ACE-inhibitor use and long-term risk of renal failure in diabetes. *Kidney Int* 2006;69:913-9.
- Hamming I, Goor H, Navis GJ. ACE inhibitor use and the increased long-term risk of renal failure in diabetes. *Kidney Int* 2006;70:1377-8.
- Woo KT, Lau YK, Wong KS, Chiang GS. ACEI/ATRA therapy decreases proteinuria by improving glomerular permselectivity in IgA nephritis. *Kidney Int* 2000;58:2485-91.
- Vleming LJ, Van Kooten C, Van Dijk M, Hollander AMJ, Pape E, Westendorp GJ, et al. The D-allele of the ACE gene polymorphism predicts a stronger antiproteinuric response to ACE inhibitors. *Nephrology* 1998;4:143-9.
- Kirschbaum BB. Analysis of reciprocal creatinine plots in renal failure. *Am J Med Sci* 1986;291:401-4.
- Woo KT, Lau YK, Wong KS, Chan CM. ACE inhibitor use and the long-term risk of renal failure in diabetics. *Kidney Int* 2006;70:1376-7.
- Hamming I, Navis G, Kocks MJ, van Goor H. ACE inhibition has adverse renal effects during dietary sodium restriction in proteinuric and healthy rats. *J. Pathol* 2006;209:129-39.
- Woo KT, Lau YK, Choong LHL, Zhao Y, Tan HB, Fook-Chong S, et al. IgA nephropathy: effects of clinical indices, ACEI/ATRA therapy and ACE gene polymorphism on disease progression. *Nephrology* 2002;7:S166-S172.
- Jacobsen PK, Tarnow L, Parving HH. Time to consider ACE insertion/deletion genotypes and individual renoprotective treatment in diabetic nephropathy? *Kidney Int* 2006;69:1293-5.
- Yong D, Qing WQ, Hua L, Kan JJ, Xi CJ, Jin QQ, Chao SH. Association of angiotensin I-converting enzyme gene insertion/deletion polymorphism and IgA nephropathy: a meta-analysis. *Am J Nephrol* 2006;26:511-8.
- Wiwantitkit V. Angiotensin-converting enzyme gene polymorphism is correlated to the progression of disease in patients with IgA nephropathy: a meta-analysis. *Ren Fail* 2006;28:697-9.
- Ng DP, Tai BC, Koh D, Tan KW, Chia KS. Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects. *Diabetologia* 2005;48:1008-16.
- Seki N, Hashimoto N, Suzuki Y, Yagui K, Saito Y. Differential effects of RAS inhibitors associated with ACE gene polymorphisms in type 2 diabetic nephropathy. *Diabetes Res Clin Pract* 2006;72:135-41.
- So WY, Ma RC, Ozaki R, et al. Angiotensin-converting enzyme (ACE) inhibition in type 2, diabetic patients – interaction with ACE insertion/deletion polymorphism. *Kidney Int* 2006;69:1438-43.
- Han SY, Kwon YJ, Jo SK, Shin JH, Cha DR, Cho WY, et al. ACE gene polymorphism and renal responsiveness to ACE inhibitors in IgA nephropathy patients. *Korean J Intern Med* 2000;15:13-8.
- Hashimoto N, Maeshima Y, Satoh M, Odaware M, Sugiyama H, Kashiwara N, et al. Overexpression of angiotensin type 2 receptor ameliorates glomerular injury in a mouse remnant kidney model. *Am J Physiol Renal Physiol* 2004;286:F516-F525.