

Original Research Article

Effect of Dual Blockade of Renin-Angiotensin Aldosterone System on Proteinuria in Patients with Diabetic Nephropathy and Advanced Azotemia

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Abstract

Purpose: To investigate the dual effect of angiotensin blockade by irbesartan and enalapril on proteinuria in diabetic patients with azotemia.

Methods: Patients with diabetes of > 5 years duration, proteinuria at a nephrotic level and serum creatinine > 1.5 mg/dL were enrolled in the study. Forty-five enrolled patients were divided into three groups, those receiving enalapril, irbesartan, or enalapril plus irbesartan, respectively, over a period of 24 weeks. Urinary protein excretion and serum level of albumin, creatinine, potassium were measured before and after treatment

Results: In patients receiving enalapril, irbesartan, and both drugs concomitantly, mean urinary protein excretion level decreased significantly at the end of 6 months from 6.46 ± 4.66 to 3.36 ± 1.60 , 5.89 ± 5.34 to 3.22 ± 1.72 and 5.99 ± 3.77 to 2.10 ± 2.22 g/day, respectively ($p = 0.001$). Decrease in proteinuria in the group receiving the combined therapy was more significant than the other two groups ($p = 0.025$). During the period of therapy, serum albumin increased and mean arterial pressure decreased significantly ($p = 0.02$ and $p = 0.002$, respectively) but serum creatinine and potassium and creatinine clearance values showed insignificant increases ($p = 0.28$ and $p = 0.57$, respectively).

Conclusion: The combined use of enalapril and irbesartan, in patients with diabetic nephropathy associated with azotemia, is more effective in decreasing proteinuria without causing any substantial increase in serum potassium levels. The combined use of these two drugs shows a more pronounced anti-proteinuric effect.

Keywords: Angiotensin-converting enzyme inhibitor, Angiotensin receptor blocker, Diabetic nephropathy, Azotemia, Proteinuria, Aldosterone, Renin, Blood pressure

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INTRODUCTION

Hypertension is a risk factor that exacerbates all vascular complications of diabetes and strongly influences the prognosis of the disease. Therefore, controlling concomitant hypertension in diabetic patients is at least as important as the control of diabetes [1].

In patients with diabetic nephropathy, proteinuria cannot be avoided and, if it lasts for a long period, the rapid development of end-stage renal failure is inevitable due to damaging effects of proteinuria on glomerules and tubules. In addition, hypoalbuminemia, resulting from proteinuria, is an important cause of mortality

and morbidity in patients with diabetic nephropathy [2]. In recent years, it has been demonstrated in several studies that anti-hypertensive drugs belonging to the classes of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II (ATII) receptor blockers, reduce proteinuria and exert renoprotective effects, in particular, by decreasing glomerular filtration rate (GFR) as well as by lowering blood pressure [2]. Recently, combination therapy of ACEIs and ATII receptor blockers has been considered in order to reduce proteinuria in patients with diabetic nephropathy. The main rationale for this combination is that the formation of ATII is not completely inhibited by ACEIs and that ATII receptor blockers can completely inhibit the effects of ATII resulting from by pathways [2]. Beneficial effects expected from both single and combined use of ACEIs and ATII receptor blockers in patients with diabetic nephropathy are reduced proteinuria as well as lowered blood pressure [3]. The effects of ACEIs as well as ATII receptor blockers on proteinuria and renal function in patients with mild to moderate renal failure and azotemia is a recent area under focus.

The principal objective of the present study was to test whether a difference exists between ACEIs and ATII receptor blockers with respect to their ability to delay impairment of renal function by reducing proteinuria in patients with diabetic nephropathy with azotemia. This study also examined the safety of combination therapy.

EXPERIMENTAL

A total of 45 patients, including 22 women and 23 men, were included in the clinical study. They were hospitalized in either the Clinics of Internal Medicine or presented to outpatient clinics of Internal Medicine of the Medical School of Ataturk University. The study was approved (2004-6/3) by the Medical School of Ataturk University Ethics Committee according to the Declaration of Helsinki and each subject gave informed consent to participate.

Patient selection and study design

Study participants had a diagnosis of diabetic nephropathy due to type 2 diabetes mellitus (DM), or were being followed for this diagnosis. Patients with creatinine levels above 1.5 mg/dL and with proteinuria of a nephrotic level (proteinuria \geq 3.5 g/day) were enrolled in the study. Their average age was 52.5 ± 4.4 years (range: 43 - 74 years) and their duration of diabetes was 7.2 ± 2.6 (5 - 14) years. Selected

demographic and clinical parameters of the 45 patients who underwent baseline testing are shown in Table I.

The patients included in the study were divided into three groups; the first group was given enalapril 10 mg/day, the second group irbesartan 300 mg/day, and the third group enalapril 10 mg/day plus irbesartan 300 mg/day. Known diabetes duration and oral antidiabetic medications in use by the patients were recorded. The effects of the drugs on proteinuria, electrolytes, renal function tests and mean arterial pressure were compared. Patients without secondary hypertension, with duration of diabetes greater than five years, a serum creatinine level above 1.5 mg/dL, proteinuria of nephrotic level, a body mass index below 30, and with diastolic blood pressure not exceeding 100 mmHg were included in the study.

Blood pressure measurement

Arterial tension values of the patients were measured with an ERKA sleeve sphygmomanometer. Patients rested 15 minutes prior to measurement. Measurements were taken twice from the right arm of sitting patients at intervals of five minutes. The results of the two measurements were averaged. Mean arterial pressure (MAP) was calculated in the patients according to the following formula: diastolic pressure + $1/3$ (systolic pressure - diastolic pressure).

Biochemical specimen collection

Morning venous blood samples were taken from the patients following 10 to 12 h of fasting and biochemical parameters were studied. Fasting blood glucose (FBG), sodium (Na), potassium (K⁺), uric acid (UA), blood urea nitrogen (BUN) and creatinine were routinely studied in the central biochemical laboratory of our hospital.

During the study period, relevant biochemical parameters of the blood samples and the level of proteinuria were monitored once in two months starting from day 15. Following initiation of drug treatment, renal function, especially serum electrolytes, were monitored closely. For proteinuria determination in urine, the result based on protein (g/dL) and creatinine (g/dL) values in the first morning urine sample and urinary protein/creatinine ratio (U P/Cr) were considered as g/g proteinuria. For each patient, these values were determined twice every other day and the mean of these results were taken.

Creatinine clearance and proteinuria measurement

Estimated creatinine clearance (eCrCl) was calculated using Cockcroft-Gault equation $[(140 - \text{age}) \times \text{weight} / 72 \times \text{serum creatinine}] \times 0.85$ for female. The patients received a diet containing 0.8 g/kg of protein and 100 mmol of sodium. During the study period, therapies that might have changed the efficacy of the treatments (e.g., diuretic and albumin use) were avoided.

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Statistical analysis

Arithmetic means and standard deviations of the values obtained at the end of the study were calculated. These values were presented as mean \pm standard deviation. SPSS for Windows, version 11.5 (Chicago, Illinois, USA) was used for statistical analysis. In-group changes between pre- and post-treatment values were calculated separately by Wilcoxon-rank test in both groups. Post-treatment values were calculated separately by Wilcoxon-rank test in both groups. Linear regression analysis was used to determine factors affecting differences in proteinuria. age, sex, differences in MAP, systolic and diastolic blood pressure, creatinine clearance (CrCl), serum creatinine (sCr), potassium, sodium, albumin and medications were included as independent variables. Kruskal-Wallis variance analysis was used to determine differences in

therapeutic outcomes among the three groups. Relationships between numeric variables were tested using the Spearman correlation analysis. A statistical significance level of $p < 0.05$ was used in this study.

RESULTS

MAP, SBP, DBP, sCr levels, eCrCl, serum albumin levels and urinary protein/creatinine ratio, as well as age and sex distribution were not significantly different among these three groups at baseline ($p > 0.05$) (Table 1).

Proteinuria and serum albumin levels in the study groups

As seen in Table II; at month six, proteinuria levels in the patients receiving enalapril, irbesartan and the combination treatment had decreased from 6.46 ± 4.66 to 3.36 ± 1.60 g/day; 5.89 ± 5.34 to 3.22 ± 1.72 g/day, and 5.99 ± 3.77 to 2.10 ± 2.22 g/day, respectively ($p = 0.001$). When the therapeutic efficacy at month 6 was evaluated based on proteinuria levels, it was found that there was no significant difference between enalapril and irbesartan groups ($p = 0.56$). However, decrease of proteinuria in the group receiving combined treatment was found to be more significant than the other two groups (Kruskal-Wallis, $p = 0.025$, mean differences in enalapril, irbesartan and combination groups 3.1, 2.67 and 3.89 respectively). Multinomial linear regression analysis showed no relationship between proteinuria and the level of decreases of MAP or the other investigated variables (ANOVA: $F = 0.996$, $p = 0.470$).

Serum albumin levels in the patients receiving enalapril or irbesartan increased from 3.22 ± 0.17 to 3.40 ± 0.54 g/dL and 3.17 ± 0.57 to 3.42 ± 0.56 g/dL, respectively, over the 6 months of the study.

Table 1: Demographic profile and clinical characteristics of patients

Parameter	Enalapril	Irbesartan	Combination	P-value
Sex (female/male)	8/7	7/8	7/8	NS
Age (years)	51.6 \pm 4.5	53.2 \pm 3.4	52.7 \pm 3.8	NS
MAP (mmHg)	103.53 \pm 9.04	106.46 \pm 10.3	105.26 \pm 4.89	NS
SBP (mmHg)	145.33 \pm 14.0	146.67 \pm 14.4	147.1 \pm 10.2	NS
DBP (mmHg)	84.0 \pm 9.1	85.6 \pm 9.02	84.33 \pm 5.6	NS
sCr (μ mol/L)	191.84 \pm 48.4	197.12 \pm 66.9	189.2 \pm 57.2	NS
eCrCl (ml/min)	38.39 \pm 6.8	38.80 \pm 11.99	41.23 \pm 10.4	NS
Serum albumin (g/dL)	3.22 \pm 0.17	3.17 \pm 0.57	3.23 \pm 0.45	NS
U P/Cr (g/g)	6.46 \pm 4.66	5.89 \pm 5.34	5.99 \pm 3.77	NS

eCrCl: estimated creatinine clearance, DBP: diastolic blood pressure, SBP: systolic blood pressure, sCr: serum creatinine, MAP: mean arterial blood pressure, NS: not significant, U P/Cr: urinary protein/creatinine ratio

These increases were statistically significant ($p = 0.03$, $p = 0.02$, respectively) (Table 2). Furthermore, treatment with enalapril plus irbesartan significantly increased the serum levels of albumin ($p = 0.02$). However, no significant difference was found in the correlation analysis performed to test for a relationship between the decrease in proteinuria level and the increase in serum albumin level in the patients receiving enalapril and irbesartan ($r = -0.96$, $p = 0.56$, $r = 0.227$, $p = 0.16$, respectively). Similarly, the increase in serum albumin level in the group receiving combination therapy was found to be independent of proteinuria ($r = 0.23$, $p = 0.15$).

Serum creatinine, CrCl, serum potassium and HbA1c levels

Increases in serum creatinine and CrCl levels of the patients receiving enalapril, irbesartan or combination therapy of enalapril and irbesartan were not significant. Pre- and post-treatment serum potassium levels in all three groups receiving treatment remained within normal limits. No patient developed hyperkalaemia requiring interruption of the treatment. Similarly, no significant differences were found with respect to serum potassium levels ($p = 0.64$) at month six for any treatment. HbA1c levels had significantly decreased in all treatment groups ($p = 0.001$) by the end of month six (Table 2).

Systolic-diastolic blood pressure and MAP levels. Significant differences were found in each of three groups (enalapril, irbesartan and combination) with respect to baseline and post-treatment systolic blood pressure values ($p = 0.001$, $p = 0.006$, $p = 0.002$, respectively) (Table 2). The same magnitude of systolic blood pressure decrease was observed in all three groups (no significant difference between groups, $p = 0.421$).

Differences were found in each of three groups with respect to baseline and post-treatment diastolic blood pressure values ($p = 0.07$, 0.028 , 0.02 for enalapril group, irbesartan and combination therapy group, respectively; Table 2). The same magnitude of diastolic blood pressure decrease was observed in all three groups (no significant difference between groups, $p = 0.528$).

A significant decrease in MAP became apparent during month two, and this decrease continued until the end of the study. In the group receiving enalapril, there was a significant decrease ($p = 0.002$) in the MAP values at month 6 when compared to pre-treatment values. Also MAP values significantly decreased in the groups receiving irbesartan ($p = 0.002$) and enalapril plus irbesartan ($p = 0.002$) at month six when compared to pre-treatment values (Table 2).

Table 2: Prior and post-treatment results for the study groups

Variable	Enalapril			Irbesartan			Combination		
	Prior	Post-	P-value	Prior	Post-	P-value	Prior	Post-	P-value
MAP (mmHg)	103.53±9.0	92.0±9.0	0.002	106.46±10.3	93.9±11.2	0.002	105.26±4.89	91.26±5.82	0.002
SBP (mmHg)	145.33±14.0	131.33±7.1	0.001	146.67±14.4	133.31±9.5	0.006	147.1±10.2	130.08±6.32	0.002
DBP (mmHg)	84.0 ± 9.1	81.0 ± 8.2	0.07	85.6 ± 9.02	83.6 ± 6.11	0.028	84.33 ± 5.6	80.09 ± 6.92	0.02
U P/Cr (g/g)	6.46±4.66	3.36±1.60	0.001	5.89±5.34	3.22±1.72	0.001	5.99±3.77	2.10±2.22	0.001
sAlb (g/dL)	3.22±0.17	3.40±0.54	0.03	3.17±0.57	3.42±0.56	0.02	3.23±0.45	3.51 ± 0.72	0.02
sCr (µmol/L)	191.84±48.4	195.36±51.0	0.5	197.12±66.9	202.4±62.5	0.22	189.2±57.2	203.28±44.9	0.28
eCrCl (ml/min)	38.39±6.8	37.7±6.7	0.06	38.80±11.99	37.5±10.6	0.11	41.23±10.4	40.76±10.0	0.57
sK ⁺ (mEq/L)	4.90±0.51	5.06±0.58	0.16	4.67±0.66	4.88±0.69	0.27	4.66±0.54	4.711±0.56	0.70
HbA1c (%)	8.90±0.60	7.10±0.22	0.001	8.8±0.73	7.21±0.36	0.001	9.02±2.7	7.32±1.17	0.001

eCrCl: estimated creatinine clearance; DBP: diastolic blood pressure; SBP: systolic blood pressure; MAP: mean arterial blood pressure; PU: proteinuria; sAlb: serum albumin; sCr: serum creatinine; sK⁺: serum potassium; U P/Cr: urinary protein/creatinine ratio

DISCUSSION

Several studies have demonstrated that ATII receptor blockers and ACEIs have anti-proteinuric and renoprotective effects in both diabetic and non-diabetic patients. It was seen that anti-proteinuric effects in both groups were not significantly different when anti-proteinuric effects in the patients receiving enalapril and irbesartan were compared. The decrease in proteinuria in the third group receiving combination therapy was found to be significant compared to other groups.

A number of studies have found that in patients with diabetic nephropathy, ACEI and ATII receptor blockers delay end-stage renal failure by slowing renal failure progression. Studies on rennin-angiotensin-aldosterone have shown that ATII is formed in several ways other than through ACE, suggesting that ACEIs fail to inhibit the rennin-angiotensin-aldosterone system [1,3]. Furthermore, ACE is not a very specific enzyme and it has several substrates other than ATII including bradykinin, substance P and neurokinins. ACEIs increase the levels of such substances [3-5]. Thus, ATII antagonists seem to provide a more specific effect than ACE inhibitors by blocking the effects of ATII at the receptor level.

The main rationale for using an ACE inhibitor combined with an ATII receptor blocker is the ACE escape mechanism, wherein ATII and aldosterone levels return to pre-treatment levels despite continuous treatment with ACEIs. Combining two drugs reduces the effect of the ACE escape mechanism while maintaining the effect of ACE inhibitors on bradykinin cleavage [2,6]. In both clinical and experimental administrations, additional effects of dual blockage have been observed on both blood pressure and neuro-humoral activation. This shows that such combination therapy inhibits the effects of ATII more prominently than fixed dose treatment with ACEIs. This study demonstrates that combination therapy reduces proteinuria to a greater extent compared with treatment with enalapril or irbesartan alone. Corresponding decreases in serum albumin and proteinuria levels were observed in the patients. This may be due to a decrease in protein loss in urine. Dietary factors may have also contributed to this outcome.

In the present study, it was observed that a decrease in proteinuria was independent of a decrease in MAP. The fact that a decrease in

proteinuria becomes apparent in later periods of treatment, whereas a decrease in blood pressure become apparent at the end of the first month of treatment, suggests that a decrease in MAP is independent of a decrease in proteinuria [7-9]. Whereas a decrease in blood pressure can occur over the short-term (within hours), it takes about 1 month for an anti-proteinuric effect to reach a maximal level. Hypertension leads to renal damage and this worsens hypertension. Now it is possible to break this vicious cycle by means of an early and efficient anti-hypertensive treatment. Such a treatment could be used in several nephropathies, especially in diabetic nephropathy.

In a study of enalapril involving patients with non-diabetic nephropathy, Apperloo *et al* [10] noted that arterial blood pressure was reduced significantly from $150 \pm 22 / 91 \pm 13$ mmHg to $132 \pm 17 / 79 \pm 10$ mmHg. In a study on hypertensive patients with nephropathy, Gansevoort *et al* gave patients either 50 or 100 mg losartan once daily. A decrease in mean blood pressure 24 h after the administration of the dose was found to be 9.2 % in the 50 mg losartan group and 10.9 % in the 100 mg losartan group. In that study, a significant difference was not found with respect to patients receiving 100 or 50 mg losartan [11].

In this study, no significant difference was found between both groups in pre-treatment and post-treatment serum creatinine levels. At the same time, no significant differences were found among any of the treatments with respect to serum creatinine levels. Reversible acute renal failure may occur on occasion in patients with diabetic nephropathy in whom proteinuria is at the nephrotic level. However, neither acute renal failure nor unexpected extreme increases in creatinine levels due to treatment were recorded in any patient during our follow-up observations. Doubling of the serum creatinine level in 583 patients with nephropathy was found to be less common in the group receiving ACEI compared to the group receiving a placebo in the "Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study" [12] An important conclusion from that study is that ACEIs do not have a renoprotective effect in patients whose daily proteinuria is below 2 g. A renoprotective effect is observed when daily proteinuria is equal to or more than three g [13].

In patients in whom diabetic nephropathy developed and GFR was reduced, it is likely that hyperkalaemia may develop due to blockage of

RAAS during treatment with both ACEIs and ATII receptor blockers. There is a higher risk of hyperkalemia in patients with more prominent nephropathy and lower GFR [14,15]. Interstitial nephritis is commonly observed in such patients during course of diabetic nephropathy further increases the risk of hyperpotassemia by means of hyporeninemic hypoaldosteronism. Using ACEIs and ATII receptor blockers requires close laboratory and clinical monitoring of these patients. In the Irbesartan Diabetic Nephropathy Trial, it was observed that only 2 % of cases resulting from use of the drug had hyperkalemia enough to stop irbesartan [16].

In a placebo-controlled study by Andersen *et al* [17] using 10/20 mg of enalapril and 50/100 mg of losartan in patients with type I diabetes mellitus, it was reported that serum potassium levels increased significantly compared to baseline levels in both treatments with 10 and 20 mg of enalapril. Serum potassium levels did not change significantly in the groups given losartan. While serum potassium levels increased in the patients receiving enalapril, it was emphasized that the difference was not significant [17]. In a study by Gansevoort *et al* involving a group of hypertensive patients, it was reported that serum potassium levels increased more prominently as a result of administration of 100 mg of losartan compared to 50 mg of Losartan. This difference was reported to be clinically significant [11]. In The Losartan Heart Failure Survival Study-ELITE trial completed in 1997, the difference between pre-treatment and post-treatment serum potassium levels was significant in patients taking captopril, and no significant difference was found in the group receiving losartan [18].

In the present study, no significant differences were found among the three groups in terms of pre-treatment and post-treatment serum potassium levels. At the same time, dangerous hyperkalemia requiring discontinuation of treatment did not develop in any patient. Reasons for no development of hyperkalemia may include restricting food enriched in potassium in the diet and relatively not high doses of the drugs given. In the cases reported in the literature, serious hyperkalemia developed when rather higher doses of drugs were given such as 20 mg of enalapril or 100 mg of Losartan. Again, if these patients are receiving potassium-sparing diuretics, the resulting increases in potassium levels may be dangerous [11].

The significant difference found between pre-treatment and post-treatment HbA1c levels in this study may have been the result of several factors, including the close monitoring of patients, dietary adjustments and efficient insulin therapy.

CONCLUSION

Administration of enalapril, irbesartan and a combination of these two drugs to patients with diabetic nephropathy presenting with apparent proteinuria and azotemia results in apparent anti-proteinuric effects. This effect is independent of the anti-hypertensive effects of the drugs. No serious side effects due to treatment were observed. They are therefore an efficient option for the treatment of proteinuria and hypertension in patients presenting with apparent proteinuria.

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