Turkish Journal of Endocrinology and Metabolism, (1999) 1 : 17-22 **ORIGINAL ARTICLE**

The Effect of Losartan on Plasma Atrial Natriuretic Peptide Levels in the Diabetic Rat Model

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Increased plasma atrial natriuretic peptide levels accompany the expansion of effective plasma volume in diabetic patients. Atrial natriuretic peptide has a deleterious effect on the pathogenesis of diabetic nephropathy by increasing renal plasma flow and glomerular filtration rate. Angiotensin converting enzyme inhibitors decrease plasma atrial natriuretic peptide levels. We aimed to show the effect of losartan, a specific angiotensin II receptor antagonist, on plasma atrial natriuretic peptide levels in diabetic rat model. Thirty-six female, 10-week-old wistar rats were enrolled in the study. Diabetes was induced by 65 mg/kg streptozotocin in pH 4.5 sodium citrate buffer injection via intraperitoneal route. The rats did not receive any kind of antidiabetic treatment throughout the study period. Following the first month of diabetes induction, the rats were separated into two groups, the first group was treated with 10 mg/kg losartan via oro-gastric lavage and the second served as a diabetic control group. Following the second month of diabetes induction, including the one-month treatment period, atrial natriuretic peptide levels were studied in trasylol and EDTA treated plasma samples. Six healthy and age- matched rats were also studied as a healthy control group.

Plasma atrial natriuretic peptide levels in diabetic untreated rats were not different from the healthy control group (104.6±4.7 pg/ml vs. 100.7±10.8 pg/ml, respectively). In the losartan treated diabetic group, plasma atrial natriuretic peptide levels were significantly lower than the diabetic control group (93.2±4.4 pg/ml, $p<0.01$), but not different from the healthy controls. As a result, treatment with angiotensin II receptor blockers decreases the plasma atrial natriuretic peptide levels in diabetic rats. A similar effect of angiotensin converting enzyme inhibitors had been shown previously. This finding may be explained by the inhibition of the renin-angiotensin-aldosterone system leading to a reduction of effective plasma volume expansion.

KEY WORDS **Losartan, atrial natriuretic peptide, diabetic nephropathy, albuminuria, glomerular filtration rate**

Introduction

Losartan is an effective antihypertensive agent, belonging to a new antihypertensive drug family: Specific angiotensin II type 1 receptor antagonists. Specific inhibition of the renin-angiotensin system at the last step of action seems to have an extra advantage compared with the non-specific effects of angiotensin converting enzyme (ACE) inhibitors on other enzymes such as kininase II. The dose dependent antihypertensive effect of losartan had been shown previously (1-4).

ACE inhibitors are the first choice drugs for the prevention and treatment of diabetic nephropathy (5-6) and found to be more effective compared with other antihypertensive agents (7). Losartan is

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also shown to be effective for the prevention of diabetic nephropathy in a limited number of experimental studies (8-9).

Glomerular hyperfiltration plays a major role in the pathophysiology of diabetic nephropathy (10-13). Treatment with ACE inhibitors, cause an increase in renal plasma flow with a significant decrease in renal vascular resistance and filtration fraction but without a change in the glomerular filtration rate (7). This effect of ACE inhibitors depends on the inhibition of the renin-angiotensin system but it is well known that systemic renin-angiotensin cascade is not activated in diabetic patients (14). Activation of local renin-angiotensin systems or an increased sensitivity to angiotensin II may be responsible (15). But there are also other humoral alterations, which seem to have an influence on glomerular hyperfiltration. An elevation in plasma atrial natriuretic peptide (ANP) levels and a correlation between elevated ANP levels and glomerular hyperfiltration had been observed in diabetic rat models (16). This finding was partly explained by effective plasma volume expansion in diabetes mellitus, which would lead to elevated ANP levels and increased glomerular filtration rate (16). The correlation between elevated ANP levels and glomerular hyperfiltration has also been reported in diabetic patients (17).

ACE inhibitors decrease plasma ANP levels in diabetic rat models and clinical studies (18-20). Thus another contributing effect of ACE inhibitors on the restoration of renal hemodynamics may be through the decrement of plasma ANP levels. The effect of losartan on plasma ANP levels has not been reported in the current literature.

Losartan will be an alternative drug for the treatment of diabetic renal disease. The effect of losartan on renal hemodynamics must be evaluated before a clinical recommendation. The aim of this study is to evaluate the effect of losartan on albuminuria, glomerular filtration rate, and mainly plasma ANP levels in the streptozotocin-diabetic rat model.

Methods

Thirty-six female, 10-week-old Wistar rats were enrolled in the study. Diabetes was induced by 65 mg/kg streptozotocin in pH 4.5 sodium citrate buffer injection via intraperitoneal route. Twenty-four hour urine samples were collected in individual metabolic cages before the administration of streptozotocin. During the study period, rats were kept in light (12 hours light, 12 hours dark) and temperature (25°C) controlled rooms in the Marmara University Animal Studies Laboratory and fed on standard rat food containing 20% protein. None of the groups received any kind of antidiabetic therapy throughout the study period.

Following the first month of diabetes induction, rats were separated into two groups. The first group was treated with 10 mg/kg losartan via oro-gastric lavage (4) and the second served as a diabetic control group. At the end of the first month, before the beginning of the treatment period and at the end of the second month, after one month of treatment, 24 hour urine samples were collected for the determination of albumin and creatinine excretion. During the 8 weeks study period, 10 rats in the diabetic control group and 5 rats in the losartan group died. Basal and pretreatment values of these rats were not included in the final analysis.

At the end of the second month blood samples were collected by intracardiac injection for determination of serum glucose, creatinine, and plasma ANP levels, while the rats were kept under ether anesthesia. Six healthy and age-matched rats were also studied as a healthy control group.

Urinary albumin excretion was determined by immunoturbidometric assay with Urin-Pak (Immuno/Bayer, USA) kit. Urine and serum creatinine levels were measured by the Jaffe method with a calorimetric kit (Boehringer Meinheim, Germany) for Hitachi 705 autoanalyser. Glucose measurements were performed by the GOD-PAP method with a calorimetric kit (Boehringer Meinheim, Germany) for Hitachi 705 autoanalyser. Plasma ANP levels were studied in trasylol and EDTA treated plasma samples with a standard RIA kit (Peninsula Lab Inc, Canada). Measurement range was 0.1-64 pg/tube, with an interassay variation coefficient of 5.7 % and intraassay variation coefficient of 6.9 %.

Statistical analyses were performed with an IBM compatible PC by Instat II program. All of the analyses were made by non-parametric tests. Kruskal-Wallis ANOVA and Mann-Whitney U tests were used for comparative and Spearman test was used for correlation analysis. The results are expressed as mean±standard deviation.

Results

Mean fasting plasma glucose was 109.3±24.7 mg/dl at the beginning and 235±105.4 mg/dl at the end of the study. Mean fasting plasma glucose levels of the diabetic control and losartan treated group were similar (228.7±128.2 mg/dl and 237.2±134.1 mg/dl, respectively).

Urinary albumin excretion rate did not increase in the healthy control group through out the study period, being 392±120 µg/day basal, 361±277 µg/day at the 1st month, 393±263 µg/day at the 2nd month (Figure 1). Urinary albumin excretion rates were similar in the losartan and diabetic control group before the induction of diabetes (469±331 µg/day vs. 526±382 µg/day, respectively), and there was a tendency to an increase at the end of the first month without treatment (952±685 µg/day vs. 970±547 µg/day, respectively). At the end of the second month urinary albumin excretion rate was significantly elevated in the diabetic control group compared with basal values and healthy controls (1724±945 mg/day, p<0.005) while it was significantly lower in the losartan group compared with the diabetic controls (778±221 µg/day, p<0.05) but an 18% decrease compared with the pretreatment values was not statistically significant (Figure 1).

Serum creatinine levels were significantly higher in both diabetic groups compared with the healthy controls (p<0.005). Although serum creatinine levels in the losartan treatment group tended to be lower than in the untreated diabetic group, they were still significantly higher than those of the healthy controls (p<0.01). Creatinine clearance rates were not statistically different among the three groups (Table 1). There was a significant negative correlation between creatinine clearance and albuminuria levels in the whole group (r= -0.57, p<0.05).

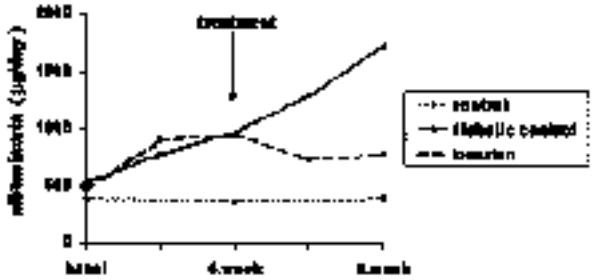

Figure 1. Urinary Albumin Excretion Rates.

Urinary albumin excretion rates were similar in the losartan and diabetic control groups before the induction of diabetes (469±331 mg/day and 526±382 µg/day, respectively) and at the end of the first month without treatment (952±685 µg/day and 970±547 µg/day, respectively). At the end of the second month urinary albumin excretion rate was significantly elevated in the diabetic control group compared with basal and control values (1724±945 µg/day, p<0.005), while it was significantly lower in the losartan group compared with diabetic controls (778±221 µg/day, p<0.05).

Table 1. Creatinine Clearance and Serum Creatinine Levels.

Groups	Creatinine clearance (ml/min)	Creatinine (mg/dl)
Diabetic	329±172	0.54±0.05
Losartan	614±482	0.49±0.13
Control	762±557	0.18±0.10

Creatinine control vs. other groups p<0.01

Plasma ANP levels of untreated diabetic rats were not different from the healthy control group (104.6±4.7 pg/ml vs. 100.7±10.8 pg/ml, respectively). For the losartan treated diabetic group, plasma ANP levels were found to be significantly lower than the diabetic control group (93.2±4.4 pg/ml, p<0.01), but not different from the healthy controls (Figure 2).

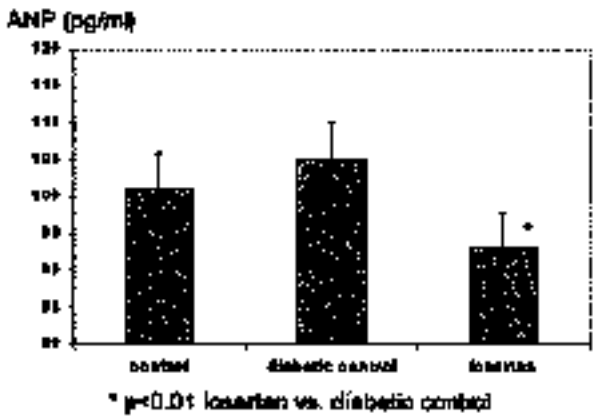

Figure 2. Plasma Atrial Natriuretic Peptide Levels.

Plasma ANP levels in untreated diabetic rats were not different from the healthy control group (104.6±4.7 pg/ml and 100.7±10.8 pg/ml, respectively). In the losartan treated, plasma ANP levels were significantly lower than the diabetic control group (93.2±4.4 pg/ml, p<0.01).

There was no correlation between plasma ANP levels and urinary albumin excretion rates, creatinine clearance rates or serum glucose levels.

Discussion

The results of this study reveal the effect of losartan on the prevention of diabetic nephropathy. A four-week period of losartan treatment stopped the progression of albuminuria in the diabetic rats. Albuminuria is the most important indicator of diabetic renal disease (10) and it is well known that control of albuminuria retards the progression of glomerular injury (21). The effect of losartan on the prevention and treatment of diabetic nephropathy has not been reported yet in diabetic patients. A limited number of rat studies have shown the effect of losartan on the prevention of diabetic albuminuria and these reports are comparable with the results of our study on this issue (8-9). ACE inhibitors are the first choice treatment for the prevention of diabetic nephropathy (5-6) and shown to be superior to other antihypertensive agents (7). As both groups of drugs target the same pathway, it is most probable that prevention of albuminuria is mainly achieved through the blockade of the renin-angiotensin system.

During the early stages of diabetic renal disease, glomerular filtration rate is significantly elevated both in IDDM and NIDDM patients (11-13). Despide being multifactorial in origin, the renin-angiotensin system is thought to be the major contributor in the development of glomerular hyperfiltration (15). Considering the findings indicating that systemic renin-angiotensin cascade is not activated in diabetic patients (14), activation of local renin-angiotensin systems or an increased response to angiotensin II is suggested as an alternative explanation (15) for the effect of ACE inhibitors on diabetic nephropathy. Atrial natriuretic peptide is another factor, which has an effect on glomerular filtration rate. Elevated plasma ANP levels with a correlation between ANP levels and glomerular filtration rate have been reported both in diabetic rat models and in diabetic patients (16-17). The expansion of effective plasma volume in diabetic patients may be a factor leading to increased plasma ANP levels. Many factors, which have been discussed elsewhere in detail (22), contribute to this volume expansion in diabetics, and the renin-angiotensin-aldosterone system is shown to be one (23). Treatment with ACE inhibitors decreases plasma ANP levels both in experimental models and in diabetic patients (18-20). Our study demonstrates a similar decrease in plasma ANP levels following a treatment period with angiotensin II receptor antagonist losartan. The effect of losartan on aldosterone had been reported previously, revealing an inhibition of angiotensin II dependent aldosterone secretion (24). The inhibition of angiotensin II dependent aldosterone secretion and control of plasma volume expansion may explain the suppressive effect of losartan on plasma ANP levels.

In this study we found similar plasma ANP levels in the untreated diabetic and healthy control groups. In a study by Ferri et al, performed on diabetic patients, elevated plasma ANP levels were found to be associated with the development of hypertension but not diabetes mellitus itself, thus normotensive diabetic patients had normal plasma ANP levels compared with non-diabetic controls (14). Although we did not measure arterial blood pressures in this study, hypertension is not a common feature of early stage diabetic nephropathy. Also in another study by Geiger et al, in a diabetic rat model , plasma ANP levels were found to be normal in the early stages of nephropathy but increased as a result of the development of hypertension and loss of renal function (18). The glomerular filtration rate of our study group was comparable with healthy controls, indicating an early stage of diabetic renal disease. Furthermore, Geiger et al demonstrated a significant decrease in plasma ANP levels without a decrease in glomerular filtration rate following a treatment period with ACE inhibitors (18). In our study we revealed a similar effect with losartan, as in the losartan treated group plasma ANP levels and urinary albumin excretion rates were found to be lower than the diabetic controls with glomerular filtration rates being similar in the two groups. Likewise we could not observe any correlation between plasma ANP levels and glomerular filtration rate or albuminuria to suggest the effect of atrial natriuretic peptide on early stage diabetic renal disease.

As a result, specific angiotensin II receptor antagonist losartan prevented the progressive increase of albuminuria in the early stage of diabetic renal disease in the diabetic rat model. Although plasma ANP levels were not increased at this early stage of diabetic nephropathy, significantly lower levels of plasma ANP were observed in the losartan treated diabetic rats. This finding may be explained by the inhibition of the renin-angiotensin-aldosterone system with losartan.

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