Kidney International, Vol. 67 (2005), pp. 2325–2329

Reduction of urinary connective tissue growth factor by Losartan in type 1 patients with diabetic nephropathy

STEEN ANDERSEN, FRANS A. VAN NIEUWENHOVEN, LISE TARNOW, PETER ROSSING, KASPER ROSSING, LOTTE WIETEN, ROEL GOLDSCHMEDING, and HANS-HENRIK PARVING

Steno Diabetes Center, Gentofte, Denmark; University Medical Center, Department of Pathology, Utrecht, The Netherlands; and University of Aarhus, Faculty of Health Science, Denmark

Reduction of urinary connective tissue growth factor by Losartan in type 1 patients with diabetic nephropathy.

Background. Connective tissue growth factor (CTGF) is an important profibrotic cytokine implicated in development of diabetic glomerulosclerosis. Urinary CTGF is reported to be significantly increased in patients with diabetic nephropathy. The present study aimed to investigate the short- and long term effects of angiotensin II receptor blockade by Losartan on urinary CTGF levels in hypertensive type 1 diabetic patients with diabetic nephropathy.

Methods. Seventy-one hypertensive type 1 diabetic patients with diabetic nephropathy were included in the study. After a washout period of 4 weeks, the patients received Losartan 50 mg, 100 mg, and 150 mg once daily in treatment periods each lasting 2 months. Thereafter, patients were followed prospectively during treatment with Losartan 100 mg o.d. with a total mean follow-up time of 36 months. At baseline, after 2, 4, and 6 months and then biannually, urinary and plasma CTGF levels [enzyme linked immunosorbent assay (ELISA) fibroGen], albuminuria (Turbidimetry), glomerular filtration rate (GFR) [51-creatinine ethylenediaminetetraacetic acid (^{51}Cr-EDTA plasma clearance)] and 24 hours blood pressure (TM2420)) were determined.

Results. Baseline levels of urinary and plasma CTGF were 7076 (5708 to 8770) ng/24 hours [geometric mean (95% CI)] and 12.7 (7.3) ng/mL [mean (SD)], respectively. Albuminuria, GFR, and arterial blood pressure at baseline were 1152 (937 to 1416) mg/24 hours, 88 (24) mL/min/1.73 m^2, and 153/80 (17/9) mm Hg, respectively. Losartan significantly reduced urinary CTGF by 21% (9 to 31) (95% CI) initially ($P < 0.05$ vs. baseline), with no further reduction after increasing dose. The sustained reduction in urinary CTGF was 22% (12 to 32) ($P < 0.05$ vs. baseline). Rate of decline in GFR during the study was 3.2 (−1.6 to 15.9) mL/min/year [median (range)]. Reduction in urinary CTGF was correlated with a lower rate of decline in GFR ($r = 0.23$, $P = 0.05$). Plasma CTGF remained unchanged throughout the study.

Conclusion. Our 3-year study demonstrates that Losartan persistently reduces urinary CTGF excretion, which is associated with a slower rate of decline in GFR.

Key words: CTGF, diabetic nephropathy, angiotensin II receptor blockade.

Received for publication September 27, 2004
and in revised form November 8, 2004
Accepted for publication January 7, 2005

Diabetic nephropathy is characterized by expansion of the extracellular matrix in the mesangium and thickening of glomerular and tubular structures [1]. Angiotensin II plays a central role in the initiation and progression of diabetic glomerulopathy, as a true cytokine that regulates cell growth, inflammation and fibrosis, contributing to progression of renal disease [2, 3]. Extracellular matrix accumulation has been attributed to hemodynamic changes associated with mechanical stress or factors such as angiotensin II and transforming growth factor-β (TGF-β) [4]. Recent studies have suggested that connective tissue growth factor (CTGF) could be a mediator of the profibrogenic effects of angiotensin II in the kidney [5]. Up-regulation of glomerular CTGF gene expression has been demonstrated in studies of experimental diabetic glomerulopathy and human renal biopsies from various renal diseases, including diabetic nephropathy [6, 7]. CTGF regulation is primarily acting downstream from TGF- β [5, 7], although recent studies have suggested TGF-β–independent induction of CTGF by hyperglycemia [8].

Animal and human studies have indicated that renin-angiotensin-aldosterone system (RAAS) blockade is likely to reduce renal expression and urinary levels of TGF-β in diabetic glomerulopathy [9–14]. Thus, blockade of the RAAS is likely to reduce glomerular scarring in diabetic nephropathy. Profibrotic factors such as CTGF and TGF-β may therefore represent supplementary therapeutic targets in treatment of diabetic nephropathy. The aim of the present study was to investigate the short- and long-term effect of increasing doses of angiotensin II receptor blockade (ARB) by Losartan on urinary levels of CTGF.

METHODS

The present study was designed to investigate the long-term renoprotective effects of Losartan in type 1 diabetic patients with diabetic nephropathy homozygous for the angiotensin-converting enzyme/insertion (I) or deletion (D) (ACE/ID) polymorphism, which have been published previously [15]. Fourteen heterozygous patients were followed by the same protocol since genotypes were unknown at inclusion in part of the patients. Data from the complete group of patients, including all ACE/ID genotypes, have not been published previously.

All antihypertensive medication was withdrawn for at least 4 weeks prior to enrollment. The patients received Losartan 50 mg, 100 mg, and 150 mg once daily in three treatment periods each lasting 2 months. Thereafter, patients were followed prospectively during treatment with Losartan 100 mg once daily with a total mean follow-up time of 36 months. Additional antihypertensive treatment (i.e., diuretics, calcium channel blockers, and alpha blockers) were given in an attempt to achieve a target blood pressure below 135/85 mm Hg. Dietary intake of protein and salt were not restricted. Blood pressure measurements and adjustment of antihypertensive medication were performed every third month. Clinical investigations were carried out every 6 months and included determination of urinary and plasma CTGF, albuminuria, glomerular filtration rate (GFR), 24-hour blood pressure, and laboratory variables.

All patients fulfilled the compliance criteria of taking more than 85% of the study medication.

All patients fulfilled the following inclusion criteria: diabetic nephropathy, GFR >60 mL/min/1.73 m^2, office blood pressure >135/85 mm Hg, and age between 18 and 70 years. Diabetic nephropathy was diagnosed clinically in patients with persistent albuminuria (>300 mg/24 hours), diabetic retinopathy, and absence of other evidence of kidney or renal tract disease [16]. Patients were excluded if they had a history of malignant hypertension, congestive heart failure, myocardial infarction, or stroke within the last 3 months. The study was performed according to the principles of the Declaration of Helsinki and approved by the Ethical Committee of Copenhagen County. All patients gave their informed consent.

Urinary CTGF was determined as the geometric mean of at least two consecutive 24-hour urine collections, completed immediately before each visit. Urinary and plasma levels of CTGF were determined by means of a sandwich enzyme-linked immunosorbent assay (ELISA), using two distinct monoclonal antibodies against the CTGF protein (FibroGen, Inc., South San Francisco, CA, USA). This assay detects both CTGF N-terminal fragments as well as the full-length CTGF protein. The sensitivity of this assay was 0.1 ng/mL, interassay and intra-assay variations were 26% and 6%, respectively [17].

Table 1. Demographic characteristics of the patients

Gender *male/female*	25/46
Age *years*	44 (9)
Duration of diabetes *years*	32 (9)
Duration of nephropathy *years*	12 (5)
Retinopathy *background/proliferative %*	19/81

Mean (SD).

Albuminuria was determined as the geometric mean of at least two consecutive 24-hour urine collections, completed immediately before each visit (Turbidimetry) (Cobas Mira Plus, Roche).

GFR was measured after a single intravenous injection of 3.7 MBq 51-creatinine-ethylenediaminetetraacetic acid (^{51}Cr-EDTA) at 8:00 a.m. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 minutes after the injection [18, 19]. The results were standardized for 1.73 m^2 body surface area, using the patient's surface area at the start of the study. The mean coefficient of variation in GFR of each patient from day to day was 4%.

Blood pressure values are based on 24-hour ambulatory blood pressure measurements performed with the Takeda TM2420, version 7 (A&D, Tokyo, Japan) device. Blood pressures were measured every 15 minutes during the day (7:00 a.m. to 11:00 p.m.) and every 30 minutes during night (11:00 p.m. to 7:00 a.m.). Values were averaged for each hour before calculating the 24-hour blood pressure.

Urinary CTGF and albuminuria were logarithmically transformed before statistical analysis owing to their skewed distribution, and are given as the geometric means (95% CI). Rate of decline in GFR is given as median (range). Clinical characteristics of participants (Table 1) are expressed as mean (SD), other data as mean (SEM). Comparisons of normally or log normally distributed parameters were performed with the use of Student *t* test. Data are analyzed by analysis of variance (ANOVA) according to a general linear model, repeated measures method. The rate of decline in kidney function was analyzed by regression lines for GFR over individually determined times during the treatment period. A P value < 0.05 was considered significant (two-tailed). Data were analyzed by SPSS, 11.5 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Baseline levels of urinary and plasma CTGF were 7076 (5708 to 8770) ng/24 hours [geometric mean (95% CI)] and 12.7 (1) ng/mL [mean (SEM)], respectively. Losartan significantly reduced urinary CTGF by 21% (9 to 31) (95% CI) initially ($P < 0.05$ vs. baseline), with no further reduction after increasing dose. The sustained