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Plasma and Urinary Endothelin-1 in Focal Segmental Glomerulosclerosis

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The kidney is an important site of endothelin-1 (ET-1) production and is particularly susceptible to ET-1 action. Infusion of ET-1 in rats induces both functional and morphological alterations in the kidneys. Increased plasma level of ET-1 has been reported in patients with chronic renal failure. However, there are still no reports on the plasma and urinary ET-1 levels in patients with focal segmental glomerulosclerosis (FSGS). In the present study, we have measured the plasma concentration and urinary excretion rate of ET-1 in 15 patients with nephrotic syndrome due to FSGS, and observed the serial changes of plasma and urinary ET-1 in nephrotic rats with FSGS, induced by repeated injection with puromycin aminonucleoside (PAN). ET-1 was measured with radioimmunoassay. The results showed that plasma ET-1 concentration in FSGS patients was significantly higher than in normal controls ($P < 0.05$), and that urinary ET-1 excretion rate was also significantly higher in FSGS patients than in normal controls ($P < 0.01$). In FSGS patients, the plasma and urinary ET-1 was significantly correlated ($P < 0.05$), and the urinary ET-1 excretion rate was significantly correlated with the amount of proteinuria ($P < 0.05$) and the glomerular sclerosing score ($P < 0.01$). In the ten rats with PAN-induced FSGS, serial examination showed a significant increase in plasma ET-1 after 8 weeks of injections, while the urinary ET-1 excretion rate showed a biphasic increase that showed a peak after 4 to 6 weeks. The same changes in plasma and urinary ET-1 levels were not observed in control rats injected with normal saline at the same frequency. Our results suggest that ET-1 may be involved in the pathogenesis of FSGS in both humans and rats. J. Clin. Lab. Anal. 15:59–63, 2001.

Key words: endothelin-1; focal segmental glomerulosclerosis; puromycin aminonucleoside

INTRODUCTION

Endothelin (ET), a very potent vasoconstrictor peptide consisting of 21 amino acids, was discovered in aortic endothelial cells by Yanagisawa et al. in 1988 (1). ET is produced not only by vascular endothelial cells but also by a variety of other cells including glomerular mesangial cells (2). The kidney is one of the most important organs for ET research because a high content of immunoreactive endothelin-1 (ET-1) is present in that organ (2–4). Elevated plasma and urinary ET-1 levels have been reported in patients with chronic renal failure (2). ET-1 has been suggested to be involved in renal disease progression because a three-week treatment with an ET(A) receptor blocker effectively prevents the progression of renal failure in uremic rats (5).

An increase in urinary ET-1 excretion rate has been found in patients with mesangial proliferative glomerulonephritis (6), and it has been suggested that ET-1 acts on mesangial cells to cause vasoactive changes which might ultimately contribute to the development of glomerulosclerosis. Focal segmental glomerulosclerosis (FSGS) is the prerequisite for renal failure; however, there have been no reports on the changes of plasma and urinary ET-1 levels in patients with FSGS. Therefore, in the present study we wanted to measure the plasma and urinary ET-1 in 15 patients with primary focal segmental glomerulosclerosis (FSGS), and observe the serial changes of both plasma and urinary ET-1 in FSGS rats receiving repeated injections of puromycin aminonucleoside (PAN). The purpose of studying the changes of ET-1 in PAN-injected rats is that these rats can be controlled in a rather uniform condition that is impossible in human studies.

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MATERIALS AND METHODS

Materials

Our study included 15 patients with primary FSGS and 15 normal controls. The 15 FSGS patients included 8 males and 7 females with a serum creatinine concentration of 1.10 ± 0.17 mg/dl, and a mean age of 35 ± 7 years old. All the FSGS patients were in the nephrotic range of proteinuria (6.5 ± 2.7 gm/day) during the study. The normal controls included 7 males and 8 females, with a mean age of 29 ± 10 years old.

Venous blood and urine from patients and controls was collected in standard tubes containing 5 mM ethylenediaminetetraacetic acid (EDTA). Following centrifugation (600*g* × 10 min, 4°C), plasma was stored at –80°C until assay. All biochemical analysis was done immediately after blood collection. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated.

The study was also carried out in Wistar rats that received repeated subcutaneous injections of PAN. PAN was injected subcutaneously 20 mg/kg body weight weekly during the first 3 weeks and subsequently 10 mg/kg body weight every other week (7). Ten rats were sacrificed after 12 weeks of PAN injection, and used for study after confirmation of FSGS. The 10 rats that received injections with normal saline at the same time as those injected with PAN were sacrificed as normal controls. The histological diagnosis of both humans and rats were based on studies of the tissue by light, electron, and immunofluorescent microscopy.

Tissue Processing and Scoring of Glomerulosclerosis

Both kidney tissues obtained from humans and rats were immediately fixed in 10% neutral buffered formaldehyde overnight, and then dehydrated by alcohol, embedded in paraffin, and stained with periodic acid schiff for light microscopy. The tissues were also immediately fixed in 2.5% glutaldehyde and 1% OsO4, dehydrated in graded alcohol, and embedded in Spur Resin for electron microscopy. The glomeruli demonstrating sclerosis were counted and separately scored from 0 to 4 according to the percentage of glomeruli involved (0% = 0, 1–25% = 1, 25–50% = 2, > 50% = 3, and global sclerosis = 4). We counted 25 glomeruli to get the final score, with a range from 0 to 100 for each patient.

Radioimmunoassay for ET-1

The immunoreactivity of supernatant was determined by a specific ET-1 radioimmunoassay (RIA, Peninsula Laboratories, Inc., Belmont, CA) after extraction. The supernatant was applied to a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) and eluted with 5 ml 60% acetonitrile in 0.1% trifluoroacetic acid. The eluate was lyophilized and reconstituted for RIA. The polyclonal antibody we used cross-reacted with the proteolytic product of ET-1, the big ET-1 (17%), and the other two ET isoforms, the ET-2 (7%) and ET-3 (7%). The recovery rate of ET-1 was measured by adding 10,000 cpm of radiolabeled ET-1 to the medium, which was also applied to the Sep-Pak C_{18} column and eluted with the same procedure as that of supernatants. The recovery rate was 61.2 ± 1.2% (n = 8). The sensitivity for ET-1 RIA was 0.4 pg/tube, and the 50% intercept was 20 pg/tube. The intra-assay and interassay coefficiencies of variation were 9.7 and 10.5%, respectively, over a range of concentration between 0.1 and 64 pg/tube (8).

Statistics

All results are expressed as mean ± SEM. Two-way ANOVA with posteriority test was used to compare the serial changes of ET-1 in PAN-injected mice.

RESULTS

Glomerular Sclerosing Scores in FSGS Patients and Rats

The glomerular sclerosing score was 18.9 ± 3.4 in FSGS patients (Fig. 1), which was significantly higher than that of normal controls (P <0.01). The glomerular sclerosing score in FSGS rats was also significantly higher than that of control rats ($P < 0.01$).

Plasma Concentration of ET-1 in FSGS Patients

The plasma concentration of ET-1 in FSGS patients was 6.94 ± 1.32 pg/ml, which was significantly higher than that of normal controls (4.02 ± 0.37 pg/ml, $P < 0.05$, Fig. 2). The plasma concentration of ET-1 was not significantly correlated with the glomerular sclerosing score, and it was also not significantly correlated with the serum concentration of creatinine, albumin, sodium, and the amount of proteinuria and the urinary sodium excretion rate.

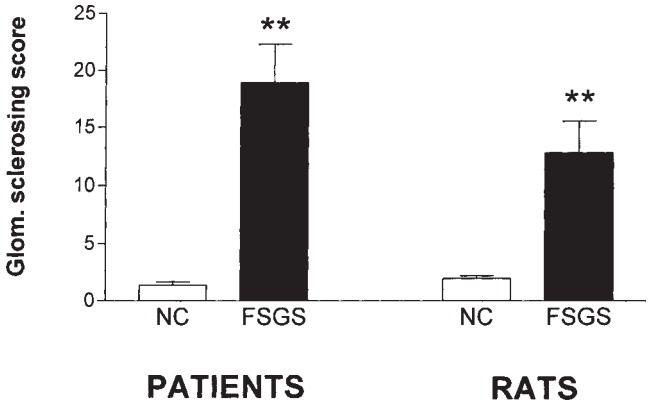

Fig. 1. Glomerular sclerosing score in 15 patients and 10 rats with focal segmental glomerulosclerosis (FSGS), compared to the kidney specimens from 5 control persons and 10 control rats, respectively. **, $P < 0.01$, compared to normal control (NC).

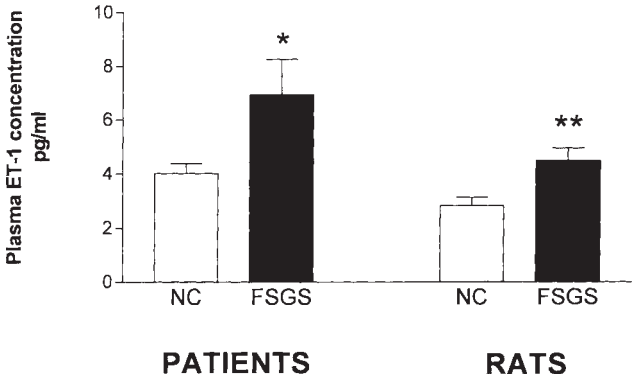

Fig. 2. Plasma concentration of endothelin-1 (ET-1, pg/ml) in 15 patients and 10 rats with focal segmental glomerulosclerosis (FSGS), compared to 15 control persons and 10 control rats, respectively. *, $P < 0.05$; **, $P < 0.01$, compared to normal controls (NC).

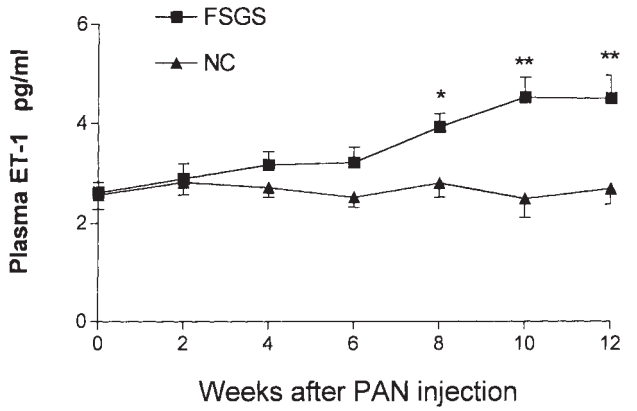

Fig. 3. Serial changes of plasma concentration of endothelin-1 (ET-1, pg/ml) in 10 rats with focal segmental glomerulosclerosis (FSGS) induced by repeated injection with puromycin aminonucleoside. *, $P < 0.05$; **, $P < 0.01$, compared FSGS to normal controls (NC) that received vehicle injection at the same time.

Urinary Excretion Rate of ET-1 in FSGS Patients

The urinary ET-1 excretion rate in FSGS patients was 115.4 ± 19.2 pg/min, which was significantly higher than that of normal controls (54.3 ± 9.7 pg/min, $P < 0.01$). The urinary ET-1 excretion rate was significantly correlated with plasma ET-1 concentration in FSGS patients ($P < 0.05$) but not in normal controls. The urinary ET-1 excretion was significantly correlated with the glomerular sclerosing score ($P < 0.01$) and the amount of proteinuria ($P < 0.05$). The urinary ET-1 excretion rate was not significantly correlated with the urinary sodium excretion rate and was also not significantly correlated with serum concentration of creatinine, albumin, and sodium.

Plasma Concentration of ET-1 in FSGS Rats

The plasma concentration of ET-1 in FSGS rats was 4.49 ± 0.46 pg/ml, which was significantly higher than that of control rats ($P < 0.01$, Fig. 2). The plasma concentration of ET-1 was not significantly correlated with the glomerular sclerosing score in either FSGS or control rats. Serial examination of plasma ET-1 concentration after PAN injection showed that there was a significant increase after eight weeks of injections (Fig. 3), and the concentration increased progressively thereafter.

Urinary ET-1 Concentration in FSGS Rats

The urinary ET-1 excretion rate in FSGS rats was 75.2 ± 8.9 pg/day, which was significantly higher than that of control rats (42.9 ± 6.5 pg/day, $P < 0.01$). The urinary ET-1 excretion rate was significantly correlated with plasma ET-1 concentration in FSGS rats ($P < 0.01$) but not in control rats. The urinary ET-1 excretion was also significantly correlated with the glomerular sclerosing score ($P < 0.01$) in FSGS rats. Serial examination showed that urinary ET-1 excretion increased early after PAN injection, which showed a peak at four to six weeks after injection. The excretion rate declined thereafter and then elevated again after 12 weeks (Fig. 4).

DISCUSSION

With this study we have demonstrated that both plasma and urinary ET-1 are increased in FSGS patients and rats. The purpose of studying the changes of ET-1 in FSGS rats is that rats can be controlled in a rather uniform condition that is impossible in human studies. Because the glomerular sclerosing score and the amount of proteinuria were significantly correlated only with urinary ET-1 excretion rate and not with the plasma ET-1 concentration, our results indicate that urinary ET-1 excretion rate may be a better indicator for glomerular damage of FSGS than plasma ET-1.

Vascular endothelium is one of the major targets for ET-1, and increased plasma ET-1 levels have been found to corre-

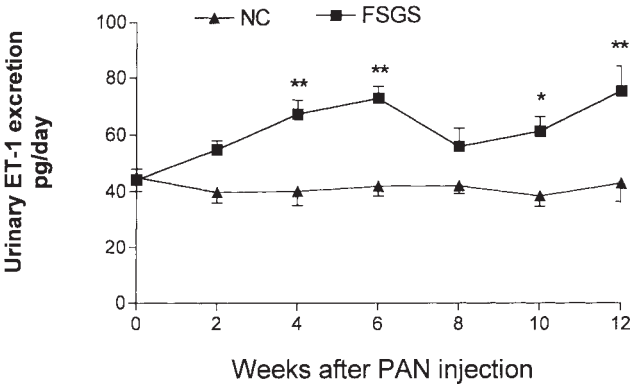

Fig. 4. Serial changes of urinary excretion rate of endothelin-1 (ET-1, pg/day) in 10 rats with focal segmental glomerulosclerosis (FSGS) induced by repeated injection with puromycin aminonucleoside. *, $P < 0.05$; **, $P < 0.01$ compared FSGS to normal controls (NC) that received vehicle injection at the same time.

late with the degree of hypertension in patients with chronic renal failure (9). Increased plasma ET-1 concentration has also been reported in nephrotic patients with normal renal function (10). Likewise, we have also demonstrated that plasma ET-1 concentrations are elevated in FSGS patients. These results indicate that elevated plasma ET-1 concentration may be a common phenomenon in patients with renal disorders. Because the kidney is not the only source for the plasma ET-1 and because plasma ET-1 concentrations are elevated in many disorders associated with renal disorders, plasma ET-1 may not be a good indicator of renal damage.

We have also examined the serial changes of plasma ET-1 in PAN-injected rats and found that plasma ET-1 is not elevated during the first few weeks of injection, while it increases later during the last few weeks. Minimal change disease (MCD) is usually found during the early weeks of PAN-injected rats (11), indicating that once plasma ET-1 is increased in MCD, the progression from MCD to FSGS may be expected. This phenomenon often occurs in human glomerular diseases.

We have further found that the urinary ET-1 excretion rate increases in FSGS patients. Increased urinary ET-1 excretion has been found in nephrotic patients with normal renal function (10), and it has also been reported to be elevated in patients with MCD but not in patients with mesangioproliferative glomerulonephritis and perimembranous nephropathy (12). Progressive renal diseases are characterized by glomerulosclerosis associated with chronic proteinuria. Numerous studies have demonstrated a correlation between proteinuria and the degree of glomerular damage, and we have further demonstrated that urinary ET-1 excretion rate is correlated with both the amount of proteinuria and the glomerular sclerosing score, indicating that urinary ET-1 could be an important marker for glomerular damage in patients with FSGS. One recent study has demonstrated that increased renal ET-1 formation (increased urinary ET-1 excretion) is associated with sodium retention and increased free-water clearance (13). However, in our study we failed to find a relationship between urinary ET-1 and either plasma or urinary sodium. Although increased urinary ET-1 excretion could be a good indicator for glomerular damage, Chu et al. could not find the significant difference between patients and controls in the daily urinary ET-1 excretion in patients with chronic renal failure (14). Therefore, urinary ET-1 may not be a good marker for renal damage once renal failure supervenes in glomerular diseases.

Serial examination of urinary ET-1 in FSGS rats show that there is a biphasic increase of the urinary ET-1 excretion rate. Although it is impossible to know the exact mechanisms for the biphasic change at present, we speculate that the change is due to the different changes of various cytokines such as transforming growth factor-β and tumor necrosis factor, in MCD and FSGS (15,16). All of these cytokines are capable of regulating ET-1 production by renal cells (17).

Most urinary ET-1 comes from the kidneys (18). Another recent study demonstrated that the renal ET-1 protein content increased in salt-sensitive hypertensive rats, and chronic administration of ET(A) receptor antagonists reduced glomerulosclerosis, indicating that ET-1 is closely associated with the development of glomerulosclerosis (19). In another study, a model of progressive disease induced by renal mass reduction, the development of glomerulosclerosis was associated with a parallel increase of renal ET-1 gene expression and the synthesis of the corresponding peptide (4). Kidneys of ET-1 transgenic mice also develop glomerulosclerosis and interstitial fibrosis (20). ET-1 also enhances mesangial synthesis of fibronectin and type IV collagen (21), both of which are major components of glomerulosclerosis, further supporting the role of ET-1 in the pathogenesis of glomerulosclerosis.

In summary, we have demonstrated that both the plasma and urinary ET-1 are increased in FSGS patients and rats, and that the urinary ET-1 excretion rate is significantly correlated with the amount of proteinuria and the glomerular sclerosing score in FSGS patients. Our results suggest that ET-1 is involved in the pathogenesis and the development of FSGS.

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