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Assessment of Urinary Endothelin-1 and Nitric Oxide Levels and Their Relationship with Clinical and Pathologic Types in Primary Glomerulonephritis

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Abstract

To determine the relationship between the urinary endothelin (ET-1), nitric oxide (NO) levels and the clinical, pathologic types of primary glomerulonephritis (GN) patients, urinary levels of ET-1 and NO were detected in 27 patients with biopsy-proven primary GN and 12 normal controls by radioimmunoassay and by copper-plated and cadmium column reduction assay, respectively. The results showed that urinary ET-1 levels in the patients with primary GN were significantly higher than in normal controls ($p<0.01$), while the urinary ET-1 levels in patients with moderate mesangial proliferation GN were significantly higher than those in patients with mild mesangial proliferation GN ($p<0.05$). Urinary ET-1 levels in patients whose clinical feature was nephrotic syndrome were found to be higher than in patients whose clinical feature was nephritic syndrome. However, urinary NO levels were to the contrary ($p<0.05$). The ratio of ET-1/NO in primary GN patients was significantly higher than that in normal controls, and it positively correlated with the 24-hour urinary excretion of protein. These results suggest that urinary ET-1 levels are related to the proliferation of mesangial cells. The imbalance between ET-1 and NO may be related to the pathogenesis of primary GN and the occurrence of proteinuria.

Key Words: Endothelin, nitric oxide, primary glomerulonephritis, urinary markers

INTRODUCTION

Endothelin (ET-1), a peptide of 21 aminoacids, is a strong and long-lasting vasoconstrictor. It is mainly synthesized and excreted by endothelial cells. It has been reported that the kidney is an important site for ET-1 mRNA expression and production, and also that it possesses ET-1 receptor. ET-1 induces mitogenesis of smooth muscle cells, as well as mesangial cells. It increases extrocellular matrix production and glomerular sclerosis.[1-3] Wolf et al. reported that the endothelin systems played an important role in the pathophysiology of different forms of glomerulonephritis (GN).[4]

NO is one of the vasorelaxing substances synthesized and released by vascular endothelial cells. It inhibits not only the production of ET-1, but also the proliferation of mesangial cells induced by mesangial contraction and growth factors.[5] In experimental glomerulonephritis, there is now good evidence that glomerular induction of NO synthesis mediates glomerular cell injury. In contrast, an intact constitutive NO release with the glomerular vasculature may be protective by decreasing glomerular capillary pressure.[6]

Urinary levels of ET-1 and NO were detected in 27 patients with biopsy-proven primary glomerulonephritis in this study. The aim was to explore the relationship between the urinary levels of ET-1, NO and the clinical and pathologic types of primary GN, and furthermore, to provide a new direction in the prevention and treatment of primary GN.

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MATERIALS AND METHODS

Subjects

Twenty-seven patients with biopsy-confirmed GN were admitted to our department from August, 1996 to April 1997. There were 18 men and 9 women,

with a mean age of 28 years (range, 15−46 years). All the patients were receiving therapy for the first time and their serum creatinine concentration was less than 133.6 μmol/L. None of them was taking steroids, immunosuppressive or nitrate-contained drugs, or converting-enzyme inhibitors. The specimens were examined both by light microscope and immunofluorescence. Each specimen included at least 5 glomeruli. Their clinical types included 19 nephrotic syndrome (proteinuria > 3.5 g/day, serum albumin < 30 g/L), 8 nephritic syndrome (proteinuria < 3.5 g/day, glomerular hematuria). Their pathologic types contained 13 mild mesangial proliferative glomerulonephritis (MsPGN), 7 moderate MsPGN, 2 membranous nephropathy (MN), 2 membranoproliferative glomerulonephritis (MPGN) and 3 focal and segmental glomerulosclerosis (FSGS). The criteria for the diagnosis of mild and moderate mesangial proliferation was 3−4 and 5−6 mesangial cells per mesangial region respectively.[7] Normal control subjects included 12 apparently healthy volunteers from the hospital staff, 6 females and 6 males, mean age of 25.5 years (range, 18−35 years).

Sample collection

Urine was collected during a complete 24-hour period from normal subjects and primary GN patients. Aliquots of urine were frozen at −70°C until assay.

Analytical methods

Levels of urinary ET-1 were measured by radioimmunoassay (RIA). RIA kit was purchased from East-Asia Immunity Technology Institute (Beijing, China). In brief, 0.1 ml standard ET-1 or sample and 0.1ml antibody (final dilution, 1 : 150,000) were preincubated at 4°C for 24 hours, followed by the addition of 0.1 ml [^{125}I] ET-1 and further incubated for 48 hours. Separation of the bound from the ligand was accomplished by the double-antibody method. The sensitivity of the ET-1 RIA was 5 pg/ml. Intra-assay and interassay coefficients of variation were less than 10% and 15%, respectively.

NO is an extremely unstable molecule and is rapidly converted in vivo and in vitro to nitrite and nitrate, respectively. Therefore, values of NO_2^- and NO_3^- in the samples have been used as an index of NO generation. NO_3^- and NO_2^- levels were detected by a copper-plated cadmium column reduction assay and by Griess reaction, respectively, as previously described.[8,9] The principle of this analysis is as follows: Solutions can be passed through a copper-plated cadmium column that reduces all the NO_3^- in the sample to NO_2^-. The concentration of NO_2^- was determined by Griess reaction. The Griess reagent consisted of one part 0.1% naphthylenediamine dihydrochloride plus one part 1% sulfanilamide in 5% concentrated phosphoric acid, mixed together and kept chilled. The color of the product dye was developed after incubation in a water bath at 60°C and its absorbance at 546 nm was detected on visible spectrophotometry. Standards of sodium nitrite and sodium nitrate, ranging from 5 to 50 nmol/ml, were analyzed daily to check column efficiency. Samples were randomized and assayed in duplicated. Data presented in the figures and tables were obtained by means of adding the NO_2^- and NO_3^- concentrations, expressed in micromoles per liter urine (μmol/L).

Statistical analysis

Data were expressed as mean standard deviation. Differences between different groups were tested using one-way analysis of variance (ANOVA) with Scheffe's F test. Comparisons between the mean values of the two groups were tested using unpaired t-test. The relationship between the ratio of ET-1/NO and the amount of proteinuria was analyzed by zero-order correlation analysis. A p value less than 0.05 was considered statistically significant.

RESULTS

Relationship between the urinary levels of ET-1 or NO and clinical types in primary GN patients

As shown in Table 1, the urinary level of ET-1 in primary GN patients was significantly higher than in normal controls ($p < 0.01$), while the urinary levels of NO in the two groups showed no significant difference ($p > 0.05$). The urinary level of ET-1 in patients whose clinical features were nephrotic syndrome was higher than in patients whose clinical features were nephritic syndrome ($p < 0.01$), while the urinary level

Table 1. Relationship between Urinary ET-1 and NO Levels in Primary GN Patients and Their Clinical Types

Clinical types	n	ET-1 (ng/24h)	NO (μmol/24h)	ET-1/NO (ng/μmol)
Nephritis syndrome	8	81.53±36.27*	467.2±233.8	0.183±0.309*
Nephrotic syndrome	19	146.5±58.09*†	298.1±161.6‡	0.628±0.336*†
Normal control	12	32.64±13.93	426.3±148.5	0.090±0.0576

* $p<0.01$, compared with normal control.
† $p<0.01$, ‡ $p<0.05$; nephritic syndrome compared with nephrotic syndrome.

Table 2. Relationship between Urinary Levels of ET-1 and NO and Pathologic Types in Primary GN

Pathologic types	n	ET-1 (ng/24h)	NO (μmol/24h)
MsPGN Mild	13	104.1±38.2*	317.9±218.6
Moderate	7	197.4±59.5	437.8±200.2
MN	2	82.67±6.01*	349.7±105.4
MPGN	2	135.8±12.6†	358.7±292.4
FSGS	3	88.39±21.32*	399.3±134.5

* $p<0.01$, † $p<0.05$; comparison with moderate MsPGN.

of NO was contradictory.

After linear correlation analysis, we found significant correlation between the ratio of ET-1/NO and 24-hour urinary excretion of protein in primary GN patients ($\gamma=0.776$, $p<0.01$). We also found that the ratio of ET-1/NO in primary GN patients was significantly higher than in normal controls ($p<0.01$), and positively related to urine ET-1 excretion ($R=0.674$, $p<0.01$) and inversely related to urinary NO excretion ($R=0.655$, $p<0.01$).

Relationship between urinary levels of ET-1 or NO and pathologic types in primary GN patients

As shown in Table 2, urinary levels of ET-1 in primary GN patients with moderate mesangial cells proliferation were higher than in patients with mild mesangial cell proliferation, MN, MPGN or FSGS ($p<0.01$, $p<0.01$, $p<0.05$, $p<0.01$ respectively), while the urinary levels of NO between the different groups showed no significant difference ($p>0.05$).

DISCUSSION

GN is mainly a kind of immunity disorder which induces renal damage by complex mechanisms. The change in kidney hemodynamics and the impaired immunity make a great contribution to the occurrence of GN. It is known that ET-1 is the strongest vasoconstrictor agent. The pathologic condition, for example ischemia of the kidney tissues, anoxia or damaged immunity, can stimulate the release of ET-1.[2] ET-1 exerts a wide range of biologic effects on the kidney, including constriction of most renal vessels, mesangial cell contraction and mitogenesis, enhancement of glomerular cell proliferation and stimulation of extracellular matrix accumulation.[1-3,10] The increased urinary ET-1 level reflected the increased synthesis and release of ET-1 by the kidney, and the urinary level of ET-1 was also related closely to the extent of impairment of kidney and sclerosis of glomeruli.[2,5,11]

The results of this study confirmed that urinary levels of ET-1 in primary GN patients increased significantly. We also found that in GN patients with moderate mesangial proliferation, the urinary levels of ET-1 were markedly higher than in patients with mild mesangial proliferation, MN, MPGN or FSGS. This suggested that in primary GN patients, the urinary excretion of ET-1 increased, and the increased urinary ET-1 levels were related to the proliferation of mesangial cells.

NO, coming from L-arginine by an inducible NO synthase (NOS), is an important effector molecule in the regulation of vascular tone, platelet aggregation, as well as inflammatory and immunologic tissue injury.[6,12] Its half-life in vivo is so short that it has an active chemical character. It is so unstable that it can react with the oxide or superoxide anion rapidly in the body and change into NO_2 and NO_3.[8]

Intrinsic NO is not only a kind of new endocellular messenger and neurotransmission, but also a strong vasorelaxing substance; it can regulate local circu-

lation of the kidney and inhibit the proliferation of mesangial cells.[13,14] Long-term inhibition of NO induces renal hypertension, increasing proteinuria and sclerosis of glomeruli.[15] Our study showed that NO derived from constitutive NOS and that inducible NOS (iNOS) may play an important role in different ways in primary GN; iNOS was localized in the mesangial cells, and endothelial NOS (eNOS) was present in glomerular endothelial cell. In glomeruli with moderate lesion in IgA nephropathy, the expression of eNOS decreased and the expression of iNOS was upregulated.[16]

The exact role of NO in primary GN is not yet fully understood. Our results showed that urinary levels of NO in patients with nephrotic syndrome was lower than in patients with nephritic syndrome. It is assumed that the mechanism is the decreased expression of eNOS caused by ischemia of kidney tissues in a progressive period of nephrotic syndrome. In contrast to ET-1, NO inhibits mesangial proliferation, but Roccatello et al. reported that local production of ET-1 was not counter-balanced by an adequate increase in NO biosynthesis in some patients with IgA nephropathy, particularly in those with established glomerular damage.[5] Our data indicated that urinary levels of NO in mild and moderate mesangial proliferative GN had no significant difference. This may be related to the decreased expression of eNOS and the upregulated expression of iNOS. We also found that urinary levels of NO between different groups had no significant difference. This correlated with Kovacs et al.,[17] who found that there was no difference in NO_2^-/NO_3^- excretion between IgA nephropathy patients and normal controls.

Long-term and heavy proteinuria induces damage to the renal structure and to renal function. Therefore, exploring the mechanism of proteinuria occurrence, and ways to decrease proteinuria becomes the key problem in the clinical treatment of GN. Remuzzi reported that enhanced renal ET-1 gene expression and urinary excretion of ET-1 was correlated with proteinuria and the degree of glomerular damage.[18] The values of urinary ET-1/NO ratio (ng/nmol) were calculated as indices of the relative balance between vasoconstrictor and vasorelaxing factors of the ET_1-NO regulation system.[19] Our data indicated that the urinary level of ET-1 in patients with nephrotic syndrome was obviously higher than in patients with nephritic syndrome, while the urine level of NO was to the contrary. We also found that the ratio of ET-1/NO in primary GN patients was significantly higher than in normal controls and significantly correlated with the amount of proteinuria. Values for the urinary ET-1/NO ratio were positively related with ET-1 excretion and inversely related with urinary NO excretion. This suggested that the imbalance of NO and ET-1 may be related to the occurrence of proteinuria in primary GN patients. The renal hemodynamic change and the different degrees of impaired immunity induced by the decrease in urine NO levels may contribute to its mechanism. The metabolic imbalance of ET-1 and NO may potentiate ET_1-mediated hemodynamic and structural effects and may represent one of the many factors which contributed to the sequence of events leading to glomerulosclerosis.

In summary, the urinary ET-1 level is related to the proliferation of mesangial cells. A certain relationship exists between the occurrence of proteinuria of primary GN and the imbalance of ET-1 and NO. In due time, we hope continued research on ET-1 and NO will help to develop a new pathway to prevention and treatment of primary GN that appropriately regulates the synthesis of NO and the administration of ET-receptor inhibitor.

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