• Original Article in English •

Expression of endothelin and nitric oxide in the renal tissue of rats with glomerulosclerosis

Ze-Quan JI, Cui-Wen HUANG, Cheng-Jie LIANG, Bo CHEN, Sheng-Qiang CHEN, Wei-Wen SUN, Ming-Hui LIN

Department of Pediatrics, Second Affiliated Hospital, Guangzhou Medical College, Guangzhou 510260, *China*

Abstract: Objective It is not known whether endothelial factor-1 (ET-1) and nitric oxide (NO) are involved in glomerulosclerosis. This study aimed at studying the changes and roles of ET-1 and NO in the process of glomerulosclerosis and the effects of the angiotensin converting enzyme inhibitor (ACEI), benazepril, and the angiotension Ⅱ receptor type Ⅰ antagonist, losartan. **Methods** Glomerulosclerosis was induced in rats by resecting one side of the kidneys and injecting adriblastine. The rats were randomly assigned into a glomerulosclerosis group (D group), a benazepril-treated glomerulosclerosis group (DB group), and a losartan-treated glomerulosclerosis group (DL group), 10 per group. Ten rats were sham-operated (Control group) and were injected with normal saline into caudal veins. After 6 weeks of benazepril or losartan administration, the mRNA expressions of ET-1 and iNOS in renal cortex were measured by reverse transcription polymerase chain reaction (RT-PCR). The protein levels of ET-1 and iNOS in renal cortex were detected by Western blotting, and the renal tissue Collagen Ⅳ and fibronectin were measured by immunohistochemistry. **Results** By the 4th week of adriblastine administration, urinary protein, serum cholesterol and blood urea nitrogen increased, while serum albumin decreased in Group D compared with those of the Control group (all $P<0.05$). RT-PCR and Western blotting demonstrated that the mRNA and protein expressions of ET-1 increased 3.58 and 2.83 times, and the mRNA and protein expressions of iNOS increased 4.28 and 3.15 times in the renal cortex of the Group D when compared with those of the Control group. The expressions of Collagen Ⅳ and fibronectin also significantly increased in Group D. After 6 weeks of benazepril or losartan treatment, the deposition of extracellular matrix in the Groups DB and DL was significantly reduced and mRNA and protein expressions of ET-1 and iNOS had decreased compared with those of the Group D. Meanwhile, the expressions of Collagen Ⅳ and fibronectin also decreased in the two treatment groups. **Conclusions** ET-1 and NO may participate in the process of glomerulosclerosis. Inhibition of ET-1 and iNOS blocks accumulation of extracellular matrix, and may avert glomerulosclerosis.

[**Chin J Contemp Pediatr, 2004, 6(4): 241－246**]

Key words: Glomerulosclerosis; Endothelin-1; Inducible nitric oxide synthase; Rat

内皮素及一氧化氮在肾小球硬化大鼠肾组织的表达及意义

纪泽泉，黄翠雯，梁成结，陈波，陈盛强，孙卫文，等 广州医学院第二附属医院儿科，广东 广州 510260

[**摘 要**] **目的** 内皮素-1(ET-1)和一氧化氮(NO)是否参与了肾小球硬化过程尚缺乏广泛的认识。本实验通过制备阿霉素肾小球硬化大鼠模型并应用血管紧张素转换酶抑制剂(ACEI)莱那普利和血管紧张素Ⅱ-Ⅰ型受体拮抗剂芦沙坦干预，观察 ET-1 和 NO 在肾小球硬化过程中的变化及作用。**方法** 大鼠随机分成假手术(对照)组(C 组)，肾小球硬化组(D 组)，肾小球硬化苯那普利治疗组(DB 组)和肾小球硬化芦沙坦治疗组(DL 组)，治疗 6 周后用 RT-PCR 分别测定肾皮质内皮素-1(ET-1)和诱导型一氧化氮合酶(iNOS)表达，用 Western blotting 测定 ET-1

[Received] February 28, 2004; [Revised] May 8, 2004
[Foundation Item] Guangzhou Committe of Science and Technology Key Program (1999－K－007－02).
[Biography] Ze-Quan JI(1966－), Male, Master, Associate Professor, Specializing in nephrology.
[Correspondence Author] Ze-Quan JI, Department of Pediatrics, Second Affiliated Hospital, Guangzhou Medical College, Guangzhou 510260 (Email:zeqj@yahoo.com.cn).

和iNOS蛋白，免疫组化测定肾组织Ⅳ型胶原(Col Ⅳ)和纤维连接蛋白(Fn)。**结果** 肾小球硬化组出现明显蛋白尿、血白蛋白下降及胆固醇上升和肾小球系膜细胞增生，细胞外基质沉积。肾皮质ET-1 mRNA和蛋白表达为对照组的3.58倍和2.83倍，肾皮质iNOS mRNA和蛋白表达为对照组的4.28倍和3.15倍，肾皮质Col Ⅳ和Fn表达也明显上调。苯那普利和芦沙坦分别治疗6周后，能明显减轻肾小球硬化的生化改变及病理改变，同时下调了ET-1、iNOS mRNA及蛋白表达，Col Ⅳ和Fn水平也降低。**结论** ET-1和NO参与了肾小球硬化进展。ET-1 and iNOS的抑制阻滞了细胞外基质的沉积，从而可以预防肾小球硬化症的发生。 **[中国当代儿科杂志，2004，6(4)：241－246]**

[关 键 词] 肾小球硬化症；内皮素-1；诱导型一氧化氮合酶；大鼠
[中图分类号] R－33 **[文献标识码]** A **[文章编号]** 1008－8830(2004)04－0241－06

There are hyperperfusion and hyperfiltration in early glomerulosclerosis. Angiotensin converting enzyme inhibitor (ACEI) can delay the development of renal insufficiency. One of its main effects is to improve the renal hemodynamic abnormality by regulating the activity of renin-angiotensin system (RAS)[1]. It is not known whether the endothelial factor-1 (ET-1) and nitric oxide (NO) participate in glomerulosclerosis. In this study, the changes and roles of ET-1 and NO in the process of glomerulosclerosis were investigated and their relationship with glomerular extracellular matrix were studied by using ACEI (benazepril) and angiotensin Ⅱ receptor type Ⅰ antagonist (losartan).

Materials and methods

Establishment of animal model, grouping and specimen collection

Forty Sprague-Dawley (SD) rats, 20 males and 20 females, provided by Animal Experiment Center of Guangzhou Medical College and weighing 160－200 g, were enrolled in this study. Glomerulosclerosis was induced by resecting one side of the kidney, combined with adriblastin injection. Before the operation, 30 rats were injected with adriblastine (6 mg/kg) into the caudel veins following intraperitoneal injection of 4 ml 2% sodium pentobarbital. Four weeks later, the rats whose 24 hour-urine protein content was more than 50 mg were defined as the ones with glomerulosclerosis. They were randomly divided into 3 groups of 10 each; a glomerulosclerosis group (D group), a benazepril-treated glomerulosclerosis group (DB group), a losartan-treated glomerulosclerosis group (DL group). Rats in the Control group (n=10) were Sham-operated and injected with normal saline into the caudal veins. The four groups were then fed in respective cages, eating and drinking freely for a period of 6 weeks. The DB Group was given gastric larvae with benazepril daily (Beijing Novartis Pharma Ltd., 10 mg/kg). The DL Group was given gastric larvae with losartan daily (MSD Pharmaceutical Co. Ltd., 40 mg/kg). The Control group and the D Group were given gastric larvae with normal saline of corresponding amounts.

Before the initial injection of adriblastine and 4 weeks later, blood and urine specimens were collected for biochemical determination. Six weeks after losartan or benazepril treatment, blood and urine specimens were collected and the kidneys were resected. Parts of them were then placed into 10% formaldehyde solution. The renal cortexes were separated from other part of the kidneys and put into liquid nitrogen for frozen saving.

Biochemical determination

Blood albumin, cholesterol, blood urea nitrogen and creatinine were determined by a Hitachi biochemical analyzer, and the amount of 24 hour-urine protein content was determined with the chromatometry of benzene 4,4′-bisphenol phenolsulfonphthalein.

Determination of ET-1 and iNOS mRNA level of renal cortex by RT-PCR

One hundred mg of fresh frozen-saved renal cortex was cut to extract total RNA in one-step fluorescein isothiocyanate aminoformamidine-Phenol-Chloroform. Its purity and content were determined by ultraviolet spectrophotometer. A260/A280 was discovered in the range of between 1.8 and 2, which suggested the content of RNA was 0.5－1.2 μg/μl. Two mg of total RNA was used to

be made into cDNA by reverse transcription with oligo (dT). The reverse transcription Test Kit was the product of Invitrogen Company, and the enzyme and amortization systems were provided by Shanghai Shengneng Bocai Company. The primers used in this study included: (1) ET-1 primer 1: 5′-AAGATCCCAGCCAGCATGGAGAGCG-3′; primer 2: 5′-CGTTGCTCCTGCTCCTCCTTGATGG-3′. (2) iNOS primer 1: 5′-GTGTTCCACCAGGAGATGTTG-3′; primer 2: 5′-CTCCTGCCCACTGAGTTCGTC-3′; (3) GAPDH primer 1: 5′-AGATCCACAACGGATACATT-3′; primer 2: 5′-TCCCTCAAGATTGT CAGCAA-3′. GAPDH was taken as inner reference to monitor RNA amount. Amplification conditions and products were follows: ET-1: 94℃ degenerated for 3 minutes, then 94℃ for 1 minute, 60℃ for 1 minute, 72℃ for 2.5 minutes, circulated 30 times totally, the final extension 72℃ for 7 minutes, the length of amplified product was 543 bp. iNOS: Denaturation at 94℃ for 2 minutes, then 94℃ for 1 minutes, 60℃ for 1 minutes, and 72℃ for 2.5 minutes, with 29 cycles; the final extension at 72℃ for 7 minutes, and the length of amplified product was 309 bp. Each amplified product was dealt by 2% agarose gel electrophoresis and was then photographed under the burdick lamp. The image of amplified strips was analyzed by a 2-dimension laser scanner, corrected by GAPDH and indicated with the ratio of absorbance.

Determination of ET-1 and iNOS protein of renal cortex with Western blotting

The total protein of the renal tissue was extracted by tissue splitting solution RIPA and the concentration of protein was determined by the modified Lowry method. Fifty μg of total protein was taken and was dealt by treated with gel electrophoresis with 15% SDS-PAGE. It was then transferred to a nitric fibrous membrane, and dyed with Ponceau to observe transferring effect and to mark the molecular weight standard of protein. Then it was sealed with TBST 4℃ containing 5% defatted milk over night. After washing membrane, either the polyclonal antibody ET-1 (rabbit-anti-rat, Santa Cruza Company, working concentration 1∶150) or the polyclonal antibody iNOS (rabbit-anti-rat, Santa Cruza Company, working concentration 1∶150) was added for hybridization respectively. Anti-rabbit IgG (working concentration 1∶150) marked by HRP was used for the hybridization of second antibody. Finally immunoblot chemiluminescence reagent was added for autoradiography. All the hybridization signals were scanned for absorbance by an IBAS-2000 image analysis system.

Determination of renal tissue Collagen Ⅳ and fibronectin with the immunohistochemistry

The renal tissue sections were dewaxed regularly, hydrated and hatched for 20 minutes with 0.1% Trypsin at 37℃. The endogenetic peroxidase was inactived by 1% hydroperoxide methanol for 8 minutes. Having been blocked by 10% goat blood serum, rabbit-anti-rat Collagen Ⅳ (Col Ⅳ) and rabbit-anti-rat fibronectin (Fn) were added to the renal tissue sections at room temperature, respectively. The sections were then treated with anti-rabbit IgG marked by HRP (Col Ⅳ immunity issue chemistry kit was the product of Wuhan Boshide Company), colored by DAB, and redyed by brazilin. Twenty continuous renal cortex sights were observed under a microscope. The staining result was classified in half quantitative analysis according to the stained area and intensity. "−" means negative staining, "1+" means lightly positive (<25%); "2+" means moderately positive (25%−50%); "3+" means severely positive (50%−75%); and "4+" means badly severely positive (>75%).

Pathological examination

The paraffin section of the renal issue was 2 μm thick, and was stained by hematoxylin and eosin. Twenty renal glomerulus of each specimen were observed double-blindly under high power objective. The renal glomerulus assault index was evaluated by calculating the mean score of the six items, i.e, the quantity of mesangial cell, mesangial base, constriction of blood capillary lumen, glomerulosclerosis, crescent and adhesion of saccule. The renal tubule interstitial sights of left-top, right-top, left-bottom, right-bottom and midst of each specimen were observed double-blindly in turn

under low power objective. The renal tubule interstitial assault index was evaluated by calculating the mean score of the following 8 items: vacuolation of renal tubular epithelial cell, renal tubule dilataltion, renal tubule atrophy, red cell cast, protein cast, interstitial edema, interstitial fibrosis, and interstitial cell infiltration[2].

Statistics analysis

The data were expressed as mean ± standard deviation ($\bar{x} \pm s$). SPSS software was used to perform the t-test and analysis of variance. The relationship among variables was evaluated with a linear correlation test.

Results

Biochemical and pathological changes of various groups

After resection of one side of the kidney and four weeks after adriblastine injection, masses of proteinuria, hypoproteinemia and hypercholesterolemia occurred in the glomerulosclerosis rats. The differences of 24-hour urinary protein and serum cholesterol levels were significant compared with those of the Control group ($P < 0.05$ or 0.01). The blood urea nitrogen and creatinine levels also rose. After six weeks of benazepril or losartan treatment, the biochemistry changes of blood and urine of the DB and DL Groups improved significantly, and the statistical differences were significant. See Table 1.

Table 1 Comparisons of biochemical parameters in the four groups ($n = 10$, $\bar{x} \pm s$)

Group	Urinary protein (mg/24 hr)	Serum albumin (g/L)	Serum cholesterol (mmol/L)	Blood urea nitrogen (mmol/L)	Serum creatinine (μmol/L)
Control	16.32±3.75	27.52±4.43	2.08±0.78	5.15±2.57	60.13±8.75
D	67.38±15.32[b]	17.53±3.83[a]	6.63±2.37[a]	9.68±5.35[a]	76.57±17.72
DB	33.87±5.72[c]	26.7±4.6[c]	3.73±1.53[c]	8.32±3.35	68.50±12.45
DL	28.45±4.36[c]	28.33±4.45[c]	3.45±1.21[c]	6.86±3.09[c]	63.37±13.08

Note: a vs the Control group $P < 0.05$; b vs the Control group $P < 0.01$; c vs Group D $P < 0.05$

The pathological results of the D Group were follows: cellular proliferation occurring in majority of mesangial cells, significant increase of extracellular matrix, part saccule adhesion, focal segment glomerular sclerosis or sclerosis of whole renal glomerulus, narrowing and emphraxis of blood capillary lumen, degeneration of renal tubular epithelial cell, multiple atrophy, part compensatory hypertrophy, protein cast, renal interstitial fibrosis, and infiltration of widespread mononuclear cell. The pathological changes of DB and DL Groups were significantly less than those of D Group, manifesting segment hyperplasia of minority of mesangial cells and extracellular matrix, seldom saccule adhesion and focal segment glomerular sclerosis, normal blood capillary lumen, no renal tubule atrophy, no interstitial fibrosis, and seldom infiltration of mononuclear cell. See Figure 1.

The changes of ET-1 and iNOS levels in various groups

The ET-1 mRNA and protein levels in D Group increased by 2.58 and 1.83 times compared with the Control group. After benazepril or losartan treatment, the ET-1 mRNA level decreased by 51% and 58% respectively. The ET-1 protein expression was also lower than that of the D Group. The iNOS mRNA and protein levels in the renal tissues were significantly higher in the D Group, by 3.28 and 2.15 times compared with the Control group. After benazepril or losartan treatment, the iNOS mRNA expression decreased by 40% and 47% respectively compared with D Group. The iNOS protein content also decreased significantly. See Table 2 and Figures 2—5.

Table 2 Expressions of the ET-1 and iNOS in the kidneys of the four groups ($n = 10$, $\bar{x} \pm s$)

Groups	ET-1		iNOS	
	ET-1 mRNA	ET-1 protein	iNOS mRNA	iNOS protein
Control	0.12±0.05	10.37±3.75	0.07±0.03	0.82±0.08
D	0.43±0.14[a]	29.34±6.18[a]	0.30±0.11[a]	2.58±0.14[a]
DB	0.21±0.12[b]	20.37±5.83[b]	0.18±0.06[b]	1.44±0.10[b]
DL	0.18±0.08[b]	18.62±6.76[b]	0.16±0.04[c]	1.08±0.09[c]

Note: a vs the Control group $P<0.01$; b vs Group D $P<0.05$; c vs Group D $P<0.01$

The changes of Col Ⅳ and Fn levels and the comparison of the pathological assault index in various groups

The expressions of Col Ⅳ and Fn in the glomerulosclerosis group increased by 1.4 and 0.75 times compared with the Control group. After treatment using benazepril or losartan for 6 weeks, the expression of Col Ⅳ decreased by 43% and 48% respectively, and the expression of Fn decreased by 34% and 36% respectively. Pathological semi-quantitative analysis showed that the glomerular aussault index (GAI) and the interstitial aussault index (IAI) in D Group increased compared with the Control group while decreased significantly in DB and DL Groups. The difference had statistical significance. See Table 3.

Table 3 Col Ⅳ and Fn levels and the pathological assault indexes in various groups ($n=10$, $\bar{x}\pm s$)

Group	Col Ⅳ	Fn	GAI	IAI
Control	9.78±3.08	14.32±5.14	0.23±0.03	0.83±0.45
D	23.47±6.27[a]	26.75±8.42[a]	4.76±2.10[b]	10.67±3.56[b]
DB	13.45±5.25[c]	18.55±5.74[c]	2.54±1.35[c]	4.13±2.42[c]
DL	12.26±4.78[c]	17.23±3.68[c]	1.87±1.23[d]	2.35±1.00[d]

Note: a vs the Control group $P<0.01$; b vs the Control group $P<0.001$; c vs Group D $P<0.05$; d vs Group D $P<0.01$

Correlation analysis

The correlation analysis showed that extracellular matrix Col Ⅳ had a positive correlation with the expressions of ET-1 and iNOS in the renal cortex ($r=0.684$, $P<0.05$; $r=0.613$, $P<0.05$); Extracellular matrix Fn also had a positive correlation with the expressions of ET-1 and iNOS in the renal cortex ($r=0.556$, $P<0.05$; $r=0.545$, $P<0.05$).

Discussion

The adriamycin glomerulosclerosis model is the classic model for investigating glomerulosclerosis[3]. One side nephrectomy plus adriamycin injection through the caudal vein were used to establish the rat glomerolosclerosis model. Significant glomerulosclerosis changes had already appeared by the end of the 4th week, which indicated that one side nephrectomy could accelerate the renal pathological changes. Its main mechanism was that one side nephrectomy changed the hemodynamic status of the renal tissue of the other side, so that there were hyperfiltration and transmembrane hypertension in the remaining single renal unit. It is suggested that hemodynamic changes had the effect of promoting the initiation and development of glomerulosclerosis. Renal hemodynamics was regulated by the dilation or contraction status of the renal arteriole. When the dilation degree of the afferent arteriole is greater than the dilation of the efferent arteriole in the glomerulus, hyperfiltration and hyperperfusion appear. This was the abnormal hemodynamic change at the early stage of glomerulosclerosis. The result of this study showed that the mRNA and protein levels of ET-1 and iNOS all increased significantly in the glomerulosclerosis group. They were consistent with the changes of extracellular matrix Col Ⅳ and Fn. After treatment using benazepril and losartan, proteinuria in rats decreased significantly; the blood albumin, cholesterol and renal function also improved significantly; the proliferation of glomerular mesangial and matrix decreased. Meanwhile, the mRNA and

protein levels of ET-1 and iNOS were down-regulated, and the Col Ⅳ and Fn levels decreased. These results were similar with those of overseas researchers[4].

NO is important in renal hemodynamic regulation. NO is mainly derived from L-arginine in the body. It is produced through the catalyse of iNOS. NO can diffuse into smooth muscle cells to activate soluble guanosine monophosphate cyclase and to stimulate the production of 3′, 5′-cyclic guanosine monophasphate. In doing so it can dilate the vascular smooth muscle. NO has a greater dilation effect on the afferent arteriole than on the efferent arteriole. NO is a signal transduction molecule. It is easy to diffuse and its half-life is only several seconds, so it is very difficult to be tested in the tissue. Kashem[5] has found in the glomerulonephritis experiment that iNOS can reflect the actual NO content under pathological circumstances. So in this study, the expression of iNOS was determined to reflect the changes of NO. The results showed that the expression of iNOS was quite low in the normal controls and that it increased significantly in rats with glonerulosclerosis. This indicates that iNOS is activated only under pathological circumstances. Some reports consider that angiotensin Ⅱ (Ang Ⅱ) can induce iNOS expression mediated by Ang Ⅱ receptor Ⅰ[6]. Other research has also shown that Ang Ⅱ can inhibit the NO release induced by IL-1[7]. It indicates that the protective effect of ACEI on the kidney has many mechanisms.

The increase of NO production will cause the compensative expression of ET-1. ET-1 and NO are not the specific indexes for glomerulosclerosis, but the hyperperfusion and hyperfiltration of glomerulus will cause the aggravation of renal pathological changes and eventually lead to glomerulosclerosis. ET is a strong vasocontractive peptide. It regulates the tension of renal vessels and mesangial cells through membrane receptor and promotes the mesangial cell proliferation and matrix formation. ET-1 can accelerate the mesangial cell DNA synthesis, extracellular matrix proliferation and interstitial fibrosis. It has been discovered that there were ETA-R and ETB-R gene expressions in glomerular mesangial cells[8]. The inflammation mediators and cytokines produced in local glomerulus can accelerate the continuous development of inflammation. ET-1 stimulates the up-regulation of intracellular adhesion molecules-1 (ICAM-1) and vascular adhesion molecules-1 (VCAM-1) expression in glomerular mesangial cells, so it can further accelerate the infiltration of inflammatory cells, enlarge the inflammation effect and cause glomerulosclerosis. Renal pathological changes and proteinuria can be improved using anti-ICAM-1 and anti-VCAM-1 monoclonal antibody[9].

Abnormal activity of RAS in local renal tissue plays a key role in the development of renal pathological changes. Angiotensin not only participates in the regulation of intraglomerular hemodynamic changes, but also is related with glomerular mesangial cell proliferation and extracellular matrix formation. It is the key factor in the development of glomerulosclerosis. Ang Ⅱ is the most important active factor in RAS and has a close relationship with glomerulosclerosis. It induces the repair, remodeling and sclerosis under pathological circumstances[10]. It has been reported that ET-1 and Ang Ⅱ can synergize each other and have double activation ability. The interaction between Ang Ⅱ and ET-1 is important in regulation of glomerular function, matrix formation and cell growth[11]. The over-expression of NO may be the main factor in the initiation of hyperperfusion and hyperfiltration at the early stage of glomerulosclerosis. The compensative over-expression of ET-1 accelerates the extracellular matrix formation, so inhibiting the production of NO may be of value in the prevention of early glomerulosclerosis. ET participates in the chronic progress of renal diseases. (All figures are on the inside front cover)

[References]

[1] Trachtman H, Futterweit S, Singhal P. Nitric oxide modulates the synthesis of extracellular matrix proteins in cultured rat mesangial cell [J]. Biochem Biophys Res Commum, 1995, 207(1): 120—125.

[2] Radford MG Jr, Donadio JV Jr, Bergstralh EJ, Grande JP. Predicting renal outcome in IgA nephropathy[J]. J Am Soc

Nephrol, 1997, 8(2): 199—207.

[3] Okuda S, Oh Y, Tsuruda H, Onoyma K, Fujimi S, Fujishima M. Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease [J]. Kidney Int, 1986, 29(2): 502—510.

[4] Bank N, Aynedjian HS. Role of EDRF (nitric oxide) in diabetic renal hyperfiltration [J]. Kidney Int, 1993, 43(6): 1306—1312.

[5] Kashem A, Endoh M, Yano N, Yamauchi F, Nomoto Y, Sakai H. Expression of inducible-NOS in human glomerulonephritis: the possible source is infiltrating monocytes/macrophagee [J]. Kidney Int, 1996, 50(2): 392—399.

[6] Zou AP, Wu F, Cowley AW Jr. Protective effect of angiotensin Ⅱ-induced increase in nitric oxide in the renal medullary circulation [J]. Hypertension, 1997, 31(4): 271—276.

[7] Kihara M, Yabana M, Toya Y, Kobayashi S, Fujita T, Iwamato T, et al. Angiotensin Ⅱ inhibits interleukin-1 beta-induced nitric oxide production in cultured rat mesangial cells [J]. Kidney Int, 1999, 55(4): 1277—1283.

[8] Kohan DE. Endothelins in the normal and diseased kidney [J]. Am J Kidney Dis, 1997, 29(3): 2—26.

[9] Kawasaki K, Yaoita E, Yamamoto T, Tamatani T, Miyasaka M, Kihara I. Antibodies against intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 prevent glomerular injury in rat experimental crescentic glomerulonephritis [J]. J Immunol, 1993, 150(3): 1074—1083.

[10] Mezzano SA, Ruiz-Ortega M, Egido J. Angiotensin Ⅱ and renal fibrosis [J]. Hypertension, 2001, 38(3 pt 2): 635—638.

[11] Gomez-Garre D, Ruiz-Ortega M, Ortego M, Largo R, Lopez-Armada MJ, Plaza JJ, et al. Effects and interactions of endothelin-1 and angiotensin Ⅱ on matrix protein expression and synthesis and mesangial cell growth [J]. Hypertension, 1996, 27(4): 885—892.

(Edited by Le ZHONG)