·Original Article in English·

Effects of Valsartan on Apoptosis and Expression of Fas and FasL in the Kidneys of Rats with Nephrotic Glomerulosclerosis Induced by Adriamycin

Zi-Ming HAN[1], *Yan XING*[2], *Hong-Wei WANG*[1], *Xiu-Ling LIANG*[2], *Jian-Hua ZHOU*[1]

1. *Department of Pediatrics, Tongji Hospital of Huazhong University of Science and Technology, Wuhan* 430030, *China*; 2. *Department of Pediatrics, the First Affiliated Hospital of Xinxiang Medical College, Xinxiang, Henan* 453100, *China*

Abstract: Objective To inquire into the effects of angiotensin Ⅱ type 1 receptor antagonist (AT1RA) valsartan on apoptosis and the expression of apoptosis correlated protein Fas and FasL in the kidneys of rats with nephrotic glomerulosclerosis induced by adriamycin. **Methods** Thirty six SD rats were randomly divided into model group, treatment group and control group. The model was established by uninephrectomy and injection of adriamycin. In the treatment group, valsartan (20 mg/kg) was delivered daily by gavage for 12 weeks. The same amount of normal saline was delivered by gavage in the control and model groups. Apoptosis was examined by means of terminal-deoxynucleotidyl transferase mediated d-UTP nick end labeling (TUNEL). Glomerular apoptotic index (GAI) and renal tubule apoptotic index (TAI) were calculated. Immunohistochemistry was utilized to detect the expression of Fas and FasL. The morphological changes were observed under optic microscope and the glomerulosclerosis index (GSI) was determined. **Results** Compared with the control group, the GSI, GAI, TAI and the expression of Fas and FasL in model and treatment groups were stronger ($P < 0.01$). Compared with model group, they decreased in treatment group ($P < 0.01$). **Conclusions** Valsartan may suppress the excessive apoptosis of kidney cells by lowering the expression of Fas and FasL so as to postpone the process of glomerulosclerosis. **[Chin J Contemp Pediatr, 2003, 5(4): 306 - 310]**

Key words: Angiotensin Ⅱ type 1 receptor antagonist; Valsartan; Adriamycin nephropathy; Apoptosis; Fas; FasL

缬沙坦对阿霉素肾病肾硬化大鼠细胞凋亡及 Fas 和 FasL 表达的影响

韩子明,邢燕,王宏伟,梁秀玲,周建华 华中科技大学同济医院儿科,湖北 武汉 430030

[**摘　要**] **目的**　探讨血管紧张素 Ⅱ1 型受体拮抗剂(AT1RA)缬沙坦对阿霉素肾病肾硬化大鼠肾脏细胞凋亡及凋亡相关蛋白的影响。**方法**　36 只 SD 大鼠随机分为模型组、治疗组和对照组。大鼠单侧肾切除加阿霉素注射诱导阿霉素肾病肾硬化模型。治疗组每日予缬沙坦(20 mg/kg)灌胃 1 次,共 12 周。对照组和模型组每日用等量生理盐水灌胃。采用 TUNEL 法检测肾脏细胞凋亡情况,计算出肾小球凋亡指数(GAI)和肾小管凋亡指数(TAI)。免疫组化法检测肾组织 Fas 和 FasL 表达。光镜下观察肾组织病理改变,并计算肾小球硬化指数(GSI)。**结果**　模型组较对照组肾小球硬化明显,GSI 高于对照组($P < 0.01$);而治疗组 GSI 较模型组降低,但仍高于对照组($P < 0.01$)。模型组和治疗组 GAI 和 TAI 较对照组显著增高($P < 0.01$);而治疗组 GAI 和 TAI 均低于模型组($P < 0.01$),但仍高于对照组($P < 0.01$)。模型组和治疗组的 Fas 和 FasL 表达较对照组亦明显增强($P < 0.01$),其中治疗组低于模型组($P < 0.01$)。**结论**　AT1RA 缬沙坦可能通过降低凋亡相关蛋白 Fas 及 FasL 在肾组织表达而抑制肾脏细胞过度凋亡,从而发挥其延缓肾小球硬化的作用。

[**中国当代儿科杂志,2003, 5(4): 306 - 310**]

[**关　键　词**] 血管紧张素 Ⅱ1 型受体拮抗剂;缬沙坦;阿霉素肾病;细胞凋亡;Fas;FasL

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[Introduction to the First Author] Zi-Ming HAN (1964 -), Male, M.D., Associate Professor.
[Correspondence Author] Zi-Ming HAN, Department of Pediatrics, the First Affiliated Hospital of Xinxiang Medical College, Xinxiang, Henan 453100, China (Email: ziminghan@sohu.com).

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Various studies have shown that the increase of apoptotic cells is significantly correlated with glomerulosclerosis[1], and the system of Fas/Fas ligand (FasL) is one of the major mechanisms to induce renal cell apoptosis[2]. The binding of Fas and its receptor FasL can induce apoptosis in several hours[3]. Angiotensin Ⅱ plays a pivotal role in the progress of glomerulosclerosis and angiotensin Ⅱ type 1 receptor antagonist (AT1RA) valsartan can prevent the binding of angiotensin Ⅱ and its receptor and postpone the process of glomerulosclerosis. However, is it achieved by partly influencing renal cell apoptosis or by the apoptosis correlated protein? Thus, the aim of this study was to inquire into the mechanism of AT1RA by observing the effects of valsartan on apoptosis and the expression of the apoptosis correlated protein Fas/FasL in the kidneys of rats with nephrotic glomeruloslerosis induced by adriamycin.

Materials and methods

Animal model and grouping

Thirty six male Sprague-Dawley rats (obtained from the Experimental Animal Center of Zhengzhou, Henan) weighing (225.08 ±31.27) g were divided into control group, model group and valsartan treatment group. Each group had 12 rats. In model group and valsartan treatment group, adriamycin (5 mg/kg) was injected by trail vein one week after left nephrectomy, while in control group the same amount of normal saline was injected after sham operation. After adriamycin or normal salin injected, valsartan (20 mg/kg) was delivered daily by gavage to the rats in treatment group, and the same amount of normal saline was given daily by gavage to the rats in the control group and model group. After 12 weeks, all the animals were sacrificed. In the course of experiment, two rats died in model group.

Materials

Immunohistochemistry kits of terminal-deoxynucleotidyl transferase mediated d-UTP nick end labeling (TUNEL), Fas and FasL were purchased from Wuhan Boster Biological Technology Ltd. The reagents included terminal deoxynucleotidyl transferase (TdT) (Promega Co. Ltd., USA), Dig-dUTP (BM Co. Ltd., Germany), biotinated anti-digoxin antibody (Sigma Chemical Co., USA), rabbit anti-Fas and anti-FasL monoclonal antibody (Calbiochem Co. Ltd., USA).

Determination of 24-hour urinary protein

24-hour urine was collected before experiment and in the 2nd, 5th, 7th, 9th, 12th week after injection of adriamycin or normal salin. Urinary protein was determined by the Coomassie light blue method.

Serum biochemistry test

Blood of rats was collected from the tail vein at 0, 6, 12 week. Serum total protein (TP), albumin (Alb), creatinine (Cr), blood uria nitrogen (BUN), cholesterol (CH) and triglycerides (TG) were determined by Beckman CX-7 automatic biochemistry analyzer.

Morphologic assessment

After rats were killed and their kidneys were removed and fixed in formalin. The tissue was embedded in paraffin in a routine fashion and processed for light microscopy. 3 μm-thick section was stained with hematoxylin-eosin (HE), Periodic acid Schiff (PAS) and PASM. Glomerulosclerosis index (GSI) was determined by Raij's method[4].

Apoptosis detection

Renal apoptosis was detected by means of TUNEL. Sections were de-waxed and re-hydrated. After being dunked in fresh 3% H_2O_2 and digested by proteinase K for 8 minutes, sections were incubated with TdT and Dig-dUTP buffer overnight at 4 ℃. The sections were treated with antidigoxin antibody and washed in TBS, followed by streptavidin-biotin complex (SABC) and diaminobenzidine (DAB). Under light microscope, cells with brown particles in the nucleus were positive cells. Positive cells of 50 glomeruli and 100 renal tubules in renal parenchyma were counted, and then the average of glomerular apoptotic index (GAI) and renal tubule apoptotic index (TAI) in every group were calculated[5].

Expression of Fas and FasL in kidney by immunohistochemistry

Enzyme digesting methods for paraffin section

were used. Sections of 4μm thick were de-waxed, re-hydrated, inacted by 3 % H_2O_2, digested by complex digestion liquid and blocked by goat blood serum. After the sections were incubated with monoclonal antibody of Fas or FasL (rabbit antirat antibody, dilution was 1∶50) respectively at 4 ℃ overnight, biotinated goat antirabbit lgG was added. They were stained with streptavidin-biotin complex (SABC) and diaminobenzidine (DAB). The positive signals were observed under optic microscope. The first antibody was replaced by phosphate-buffered saline (PBS) as negative control. The average absorbence of Fas and FasL were detected by IDA-2000 digital microgram analysis system in 10 random fields of each section (viewed at ×200 magnification).

Statistical analysis

Numerical variables were expressed with means and SD. Statistical analysis were performed by SPSS 10.0 for Windows. One-way analysis of variance (ANOVA) was used to compare the results among 3 groups. If the variances assumed were equal, LSD was used to compare differences between two groups. Otherwise, Dunnet *t* test was used. Pearson method was performed for correlation analysis.

Results

24-hour urinary protein

From the 2nd week, the levels of 24-hour urinary protein in model group and treatment group were significantly higher than those in control group ($P < 0.01$). From the 7th to the 12th week, the levels of 24-hour urinary protein in treatment group were significantly lower than those of model group ($P < 0.05$). See Table 1.

Table 1 24-hour urinary protein excretion of 3 groups in different stages ($\bar{x} \pm s$, mg)

Groups	n	2 week	5 week	7 week	9 week	12 week
Control group	12	8.43 ±0.59	8.23 ±0.97	9.73 ±0.97	10.93 ±2.32	11.63 ±1.27
Model group	10	29.80 ±3.19[a]	37.61 ±6.59[a]	47.27 ±8.08[a]	64.42 ±9.90[a]	70.13 ±15.08[a]
Treatment group	12	30.91 ±3.42[a]	35.95 ±4.90[a]	33.32 ±7.30[a,b]	32.43 ±7.96[a,b]	38.70 ±6.89[a,b]

Note: a vs control group $P < 0.01$; b vs model group $P < 0.05$

Serum biochemical indexes

In the 12th week, the levels of CH, TG, BUN and Cr in the model and treatment groups were higher than those in the control group ($P < 0.01$ or 0.05). The level of Alb in the treatment group was higher than that in the model group ($P < 0.05$), although it was lower than that in the control group, but the difference was not obvious ($P > 0.05$). See Table 2.

Table 2 Serum biochemical indexes of 3 groups in the 12th week ($\bar{x} \pm s$)

Group	n	Alb (g/L)	TP (g/L)	CH (mmol/L)	TG (mmol/L)	Cr (μmol/L)	BUN (mmol/L)
Control group	12	40.27 ±1.78	78.00 ±9.76	1.77 ±0.24	1.02 ±0.57	50.57 ±3.69	5.61 ±0.78
Model group	10	30.21 ±9.50[a]	69.42 ±13.01	6.12 ±2.09[a]	4.72 ±1.11[a]	77.92 ±16.26[a]	9.26 ±2.80[b]
Treatment group	12	37.63 ±7.07[c]	76.00 ±19.45	5.41 ±1.84[a]	4.02 ±0.86[a]	71.89 ±9.99[b]	9.42 ±2.46[b]

Note: a vs control group $P < 0.01$; b vs control group $P < 0.05$; c vs model group $P < 0.05$

Changes of renal pathology

Under optic microscope, capillary loops opened well in control group. In the model group, part of glomeruli had segmental sclerosis. Most glomeruli presented with mesangial matrix proliferation, mesangial areas expansion, and part of capillary lumen segmentally collapse and/or adhering to Bowman's capsule. In the treatment group, glomerular injuries were ameliorated and the GSI was significantly lower than that in model group, although the GSI in the model and treatment groups were all higher than that in control group ($P < 0.01$). See Table 3.

Renal cell apoptosis

In the control group, there were a few apoptoic

cells. In the model group, the apoptotic cells were significantly more than those in the control group ($P < 0.01$). See Figure 1. Most apoptotic cells can be seen in clusters of distal renal tubule, some were in proximal tubule and interstitium, while others appeared in glomeruli. In the treatment group, the number of apoptotic cells was significantly reduced compared with that of the model group ($P < 0.01$), although it was still higher than that in the control group ($P < 0.01$). See Figure 1. In the treatment group, the GAI and TAI were significantly lower than those in model group ($P < 0.01$). But in the treatment and model groups, they were higher than those in control group ($P < 0.01$). See Table 3.

Table 3 Some experimental results of 3 groups ($\bar{x} \pm s$)

Groups	n	GSI (%)	GAI (%)	TAI (%)	Absorbence of Fas	Absorbence of FasL
Control	12	47.8 ±17.7	8.6 ±3.1	9.6 ±5.0	0.29 ±0.03	0.26 ±0.02
Model	10	175.4 ±39.2[a]	60.1 ±5.6[a]	102.2 ±12.5[a]	0.52 ±0.15[a]	0.46 ±0.04[a]
Treatment	12	87.3 ±28.7[a,b]	30.6 ±3.4[a,b]	54.9 ±6.6[a,b]	0.38 ±0.03[a,b]	0.37 ±0.02[a,b]

Note: a vs control group $P < 0.01$; b vs model group $P < 0.01$

Expression of Fas and FasL in kidney

The expression of Fas and FasL in the control group was weak. FasL expressed mainly in tubular epithelial cells. The expressions of Fas and FasL in the control group were lower than those in the treatment and model groups ($P < 0.01$). In the model group, they were higher than those in the treatment group ($P < 0.01$). See Figures 2, 3 and Table 3 (All figures were on the Cover Ⅲ).

Correlation analysis

The GSI and GAI as well as TAI had a significant correlation. The correlation coefficients were 0.77 and 0.84 respectively ($P < 0.01$). The correlation coefficient of GAI and TAI was 0.929, $P < 0.01$.

Discussion

In the processes of progressive glomerulosclerosis, the renal cells gradually decrease after a short time of increase. Many studies in vivo and in vitro have suggested that apoptosis is one of the important mechanisms of the progressive loss of renal cells[1,2]. Fas and FasL belonging to the super family of tumor necrosis factor (TNF) are type Ⅰ and Ⅱ transmembrance protein respectively. The binding of Fas and FasL, or the specific antibodies can induce apoptosis. Fas and FasL widely exist in kidneys, and their expression can be detected in the renal tissue[3]. Ding[2] reported that Fas/FasL took part in the apoptosis of mesangial cells in the early stages of glomerular injury, and played a role in the processes of glomerulosclerosis. Our study found that the amount of apoptotic cells of renal parenchyma increased in the nephrotic glomerulosclerosis induced by adriamycin, and that there was a significant positive correlation between GSI and GAI as well as TAI. Meanwhile, the expression of Fas and FasL also significantly increased.

Our study showed that the degree of glomerulosclerosis, the number of apoptotic renal cells and the expression of Fas and FasL decreased after administration of valsartan, which indicated that valsartan alleviated glomerulosclerosis by suppressing apoptosis of kidney. The possible mechanisms of suppressing apoptosis by valsartan was blocking the activation of angiotensin Ⅱ on the levels of receptor, because angiotensin Ⅱ participated in apoptosis through the following pathways[6-9]. Firstly, the binding of angiotensin Ⅱ and its receptors, which can increase the influx of calcium ions via the mechanisms of signal transduction, induces apoptosis. Secondly, the glomerular hypertension can induce apoptosis of some cells. Thirdly, angiotensin Ⅱ can promote the expression of transforming growth factor-β (TGF-β) which is an inducer of apoptosis. Fourthly, angiotensin Ⅱ can induce oxidative stress and produce a vast amount of reactive oxygen species(ROS), which can induce the apoptosis of all kinds of cells. Fifthly, the accumulation of extracellular matrix (EMC) re-

sults in the absences of nutritional materials and promoting growth factors locally, which also induce apoptosis. Therefore, the blockade of angiotensin II is likely to alleviate the apoptosis and play a pivotal role in postponing the processes of glomerulosclerosis. In addition, it seems that vasartan itself affected the expression of the apoptosis correlated protein, but the mechanisms of this function have not been known. Further studies are required to elucidate whether valsartan suppresses the apoptosis of renal cells directly or by its effect on renin-angiotensin system.

From the above analysis, we can conclude that in the rats model of glomerulosclerosis induced by uninephrecomy and injection of adriamycin, the apoptosis of renal parenchymal cells increase. Valsartan may suppress the excessive apoptosis of renal cells by downregulating the expression of the apoptosis correlated protein Fas and FasL so as to postpone the processes of glomerulosclerosis.

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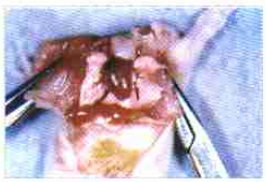
(Edited by Yan YU)

Effects of Tumor Necrosis Factor- α and I κ B α on Pulmonary Hemorrhage of Neonatal Rats Induced by Lipopolysaccharide

（正文见第 301 页）



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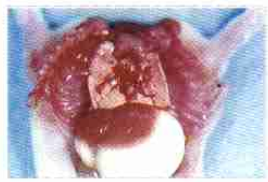

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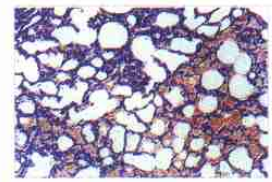

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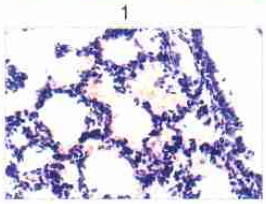

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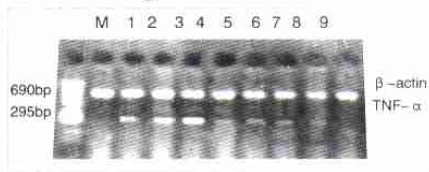

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Figure 1 The lung of normal neonatal rat

Figure 2 The lung of neonatal rats 1 h after administration of LPS

Patch-like bleeding which spread out from the hilus of the lung was observed in more than two lobes

Figure 3 The lung of neonatal rats 2 hs after administration of LPS

The diffuse bleeding was seen in every lobe in the lung

Figure 4 The lung of neonatal rats 1 h after administration of LPS

A lot of red blood cells were seen in the lung interstitial tissue and alveolar cavity (HE × 200)

Figure 5 The lung of neonatal rats 24 hs after administration of LPS

A lot of red blood cells and PMN were seen in the lung interstitial tissue and alveolar cavity (HE × 400)

Figure 6 The expression of TNF-α in the lungs of NS group and LPS groups at various time points

From left to right: M: Marker; 1. NS; 2. 0.5 h; 3. 1 h; 4. 2 h; 5. 4 h; 6. 8 h; 7. 16 h; 8. 24 h; 9. Vacuity

Figure 7 The expression of I κ B α in the lungs of NS group and LPS groups at various time points

From left to right: 1. NS; 2. 0.5 h; 3. 1 h; 4. 2 h; 5. 4 h; 6. 8 h; 7. 16 h; 8. 24 h

Effects of Valsartan on Apoptosis and Expression of Fas and EasL in the Kidney of Rats with Nephrotic Glomerulosclerosis Induced by Adriamycin

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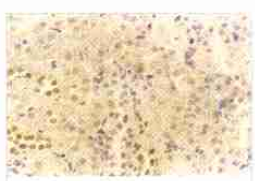

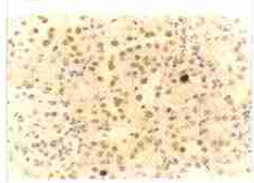

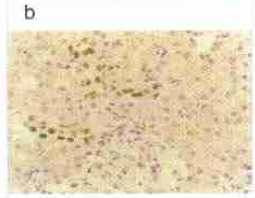

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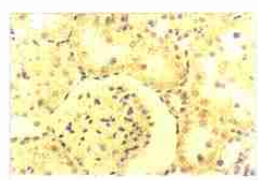

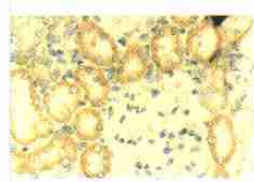

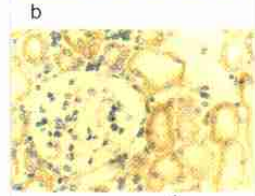

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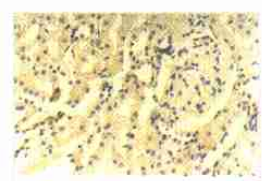

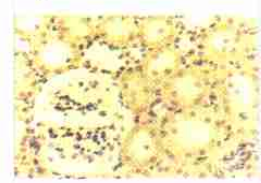

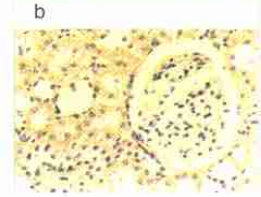

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Figure 1 Results of TUNEL (× 400)

Compared with control group, the number of apoptotic cells in model group increased significantly, and the number of apoptotic cells in treatment group was significantly lower than that in model group.

Figure 2 Immunohistochemical results of Fas (× 400)

Within the tubular and glomerular regions, expression of Fas in model group was higher than that in control group, and the expression of Fas in treatment group was weaker than that in model group.

Figure 3 Immunohistochemical results of FasL (× 400)

Within renal cortex, the expression of FasL in model group was higher than that in control group, and the expression of FasL in treatment group was weaker than that in model group.