

The effect of root of rhododendron on the activation of NF- κ B in a chronic glomerulonephritis rat model

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Abstract

Objective: We have explored the role of nuclear factor kappa B (NF- κ B) in the pathogenesis of chronic glomerulonephritis, and investigated the effect of rhododendron root on the activation of NF- κ B. **Methods:** Thirty-six Wistar rats were randomly divided into three groups: a control group, a glomerulonephritis model group and a therapy group (glomerulonephritis animals treated with the root of rhododendron). Bovine serum albumin (BSA) nephritis was induced by subcutaneous immunization and daily intraperitoneal administration of BSA. Twenty-four-hour urinary protein and serum creatinine values were measured, and renal pathology was assessed histologically by optical microscopy and electron microscopy. NF- κ B activity was determined by an electrophoretic mobility shift assay (EMSA). **Results:** Compared with the control rats, glomerulonephritis model rats exhibited a significant increase in both 24 h urinary protein and serum creatinine, and had abnormal renal histology. The administration of the root of rhododendron ameliorated these changes. NF- κ B activity in glomerulonephritis model group was greater than that in rhododendron-treated group, and NF- κ B activity was greater in both glomerulonephritis groups than in the control group ($P < 0.01$). **Conclusion:** These observations suggest that NF- κ B plays a role in the pathogenesis of chronic glomerulonephritis, and rhododendron root may attenuate renal damages by downregulating the activation of NF- κ B in this model.

Key words: glomerulonephritis; nuclear factor kappa B; rhododendron; rat model

INTRODUCTION

Within the kidney, cytokines play a central role in signaling between infiltrating leukocytes and intrinsic renal cells, orchestrating the effector responses that lead to renal damage. Glomerulonephritis results from adaptive responses that targets the kidney and leads to renal failure^[1-3]. Nuclear factor kappa B (NF- κ B) is an important transcription factor that plays a critical role in inflammatory responses and in the regulation of the body's immune system^[4,5]. The present study is aimed at exploring the role of NF- κ B in the pathogenesis of chronic glomerulonephritis, and to examine the effect

of rhododendron root on the activation of NF- κ B.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), incomplete Freund's adjuvant (IFA) and lipopolysaccharide (LPS) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Mouse anti-NF- κ B P65 monoclonal antibody and goat anti-mouse IgG-HRP were purchased from Jingmei Biotech Co. Ltd. (Shenzhen, China). SYBR Green qPCR Kits was bought from Beijing Dingxin Biotech Co. Ltd. (Beijing, China). Wistar rats were supplied by the Animal Center of Tongji Medical College, Huazhong University of Science and Technology. Root of rhododendron was a gift from Professor Luo Yongyan of Wuhan Union Hospital and was

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made into a decoction (concentration, 1 g/ml) as certified by Professor Zhang Changgong, Department of Pharmacy, Tongji Medical College.

Induction of a chronic glomerulonephritis model in rats

Thirty-six male Wistar rats (200 ± 10 g) were randomly divided into a control group, a glomerulonephritis model group and a therapy group, of 12 rats each. The method of Arisz and colleagues^[6] was used to induce chronic immune complex glomerulonephritis in both the model and therapy groups. In addition, the left kidneys were removed under chloral hydrate anesthesia. After one week, the rats were pre-immunized in footpad with a mixture of 3 mg BSA and IFA, and then were injected subcutaneously at different sites once every two weeks until the titer of serum anti-BSA antibody reached 1:16 (double immunodiffusion method), at which time daily intraperitoneal administration of 3 mg BSA (immunization) was started. Three weeks later, LPS (100 μ g) was given via intraperitoneal injection. After the intraperitoneal administration of BSA for 6 weeks, the animals were killed by cervical dislocation. The rats in the therapy group were treated with root of rhododendron extract via gastric gavage at a dose of 1.5 g/kg per day. The rats of the glomerulonephritis group were treated with the same volume of saline, starting at the time of intraperitoneal BSA administration. The left kidneys were also removed from control rats and these animals were pre-immunized with IFA, without BSA. These rats then underwent intraperitoneal administration of BSA and LPS as described above.

Biochemical index tests and renal samples collection

At the end of 4 weeks and before the animals were killed, animals were individually housed in metabolic cages and 24-hours urine samples were collected. The total urinary protein excretion was measured using a Coomassie Brilliant Blue dye method. Serum creatinine was measured using a Hitachi 7150 automatic biochemical analyzer. Kidney tissue samples were divided into 3 parts: one part was fixed with 4% paraformaldehyde, embedded in paraffin, and sections stained with hematoxylin and eosin for standard histology; one part was fixed with 2% glutaraldehyde and prepared, embedded and sectioned for electron microscopy using standard techniques; one part was stored in liquid nitrogen for subsequent molecular biological testing.

Activation of NF- κ B

An electrophoretic mobility shift assay (EMSA) was used to detect the activation of NF- κ B. The assay was

based on the fact that DNA-protein complexes migrate slower than unbound double-stranded oligonucleotides in a native polyacrylamide gel, resulting in a "shift" in migration of the labeled DNA band. The band was detected by LightShift™ Chemiluminescent EMSA kit (Pierce Biotech Inc., USA) that uses a non-isotopic method. Biotin end-labeled DNA duplex of sequence 5' -AGT TGA GGG GAC TTT CCC AGG C-3' and 3' -TCA ACT CCC CTG AAA GGG TCC G-5' containing a putative binding site for NF- κ B was incubated with the nuclear extracts. After the reaction the DNA-protein complexes were subjected to a 6% native polyacrylamide gel electrophoresis and transferred to a nylon membrane. After the transfer, the membrane was immediately cross-linked for 10 minutes on a UV transilluminator equipped with 254 nm bulbs. A chemiluminescent detection method utilizing a lumino/enhancer solution and a stable peroxide solution (Pierce Biotech Inc., USA) was used as described by the manufacturer, and membranes were exposed to x-ray from 30 seconds to 5 minutes. The bands were scanned with an Epson Expression 1600 Pro, and relative intensities were analyzed with a Gel-Pro-Analyzer 4.0.

Statistical analysis

Data are shown as mean \pm SD, and were analyzed with SPSS 12.0 software using one-way ANOVA analysis (Post Hoc Tests: LSD test). The level of significance was set at $P < 0.05$.

RESULTS

Metabolic changes

Compared with the control group, both the glomerulonephritis model group and the therapy group exhibited significant increases in the 4th and 6th week 24-hours urinary protein excretion and blood creatinine ($P < 0.01$), with the exception of the 4th week serum creatinine level in the therapy group animals. Similarly, the values of both parameters in the therapy group were significantly lower than those in the model group ($P < 0.01$) (Table 1).

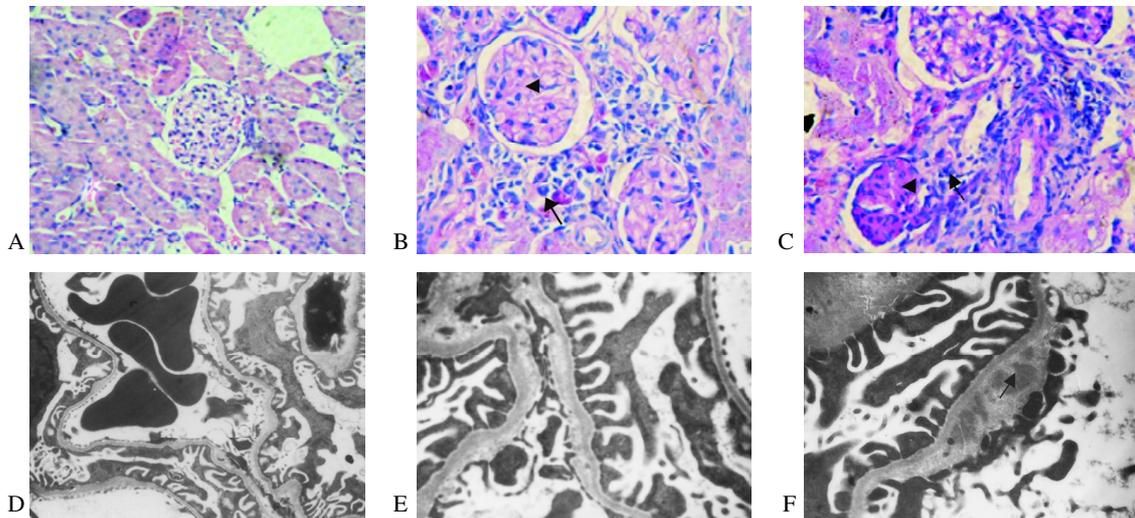
Histological changes

Optical microscopy revealed mesangial cells and mesangial matrix proliferation, inflammatory cells accumulation, and focal segmental glomerulosclerosis in the model group and in the therapy group. However, compared with the model group, these histological changes were significantly ameliorated in the therapy group. Using electronic microscopy, immune complex deposition was observed in subendothelial areas and mesangium in the model group. No obvious immune complex deposition was observed in the therapy group or in control rats (Fig. 1).

Table 1 The changes of 24-hours urinary protein excretion and blood creatinine

Index	Control group		Model group		Therapy group	
	4w	6w	4w	6w	4w	6w
24-hours urinary protein excretion(mg/24 h)	2.9 ± 0.2	3.1 ± 0.3	75.3 ± 9.1**	112.5 ± 14.7**	49.8 ± 5.6*	80.3 ± 9.6*
Serum creatinine(μmol/L)	45.5 ± 4.7	45.8 ± 5.3	49.2 ± 6.1**	58.8 ± 6.5**	46.2 ± 5.7	50.3 ± 6.2*

Compared with the control group, **P* < 0.01; the model group compared with the therapy group, #*P* < 0.01.



There was histological evidence of mesangial cells and matrix proliferation(B and C, arrowhead), and inflammatory cells accumulation(B and C, arrow) and focal segmental glomerulosclerosis were observed in the kidneys of the model group and the therapy group animals, but not the control group animals. These changes were fewer in the therapy group animals than in the model group animals. Electron microscopy revealed immune complex deposition(F, arrow) in the subendothelial areas and in the mesangium of the model animals only.

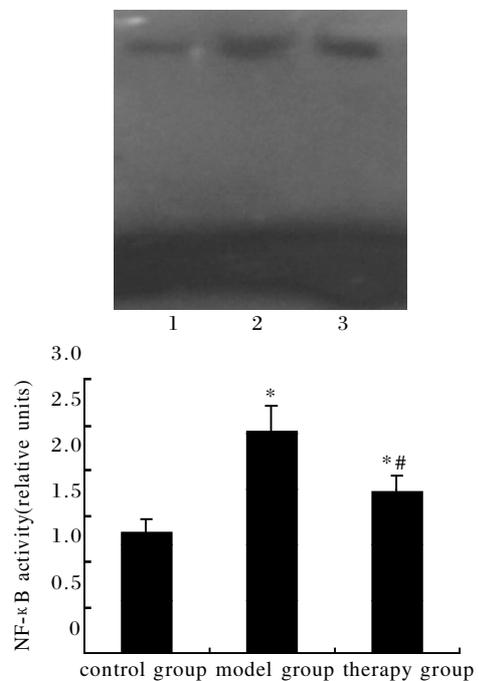
Fig. 1 Histological(A-C, 400X) and ultrastructural(D, 1,500X; E and F, 2,000X) appearance of representative sections from the kidneys of control group(A, D), therapy group(B, E) and glomerulonephritis model group(C, F) animals

Activation of NF-κ B

Activation of NF-κ B was determined by a non-radioactive gel shift assay. The NF-κ B activity was expressed as the ratio between the photodensity of NF-κ B and the photodensity of the background by densitometry analysis with the HPIAS-1000 software(Champion Image Engineering Company of Tongji Medical College affiliated to Huazhong University of Science and Technology, China). EMSA revealed that NF-κ B activity increased to 2.43 ± 0.28 relative units in model group and increased to 1.76 ± 0.19 relative units in therapy group, compared with 1.31 ± 0.15 in control rats(*P* < 0.01). And there was a significant difference between the NF-κ B activity of the modern rats and that of the therapy rats(*P* < 0.01)(**Fig. 2**).

DISCUSSION

NF-κ B is an important eukaryotic transcription factor that participates in many biological activities. In its inactive form, NF-κ B is sequestered in the cytoplasm, bound by members of the Iκ B family of inhibitor proteins. The activation of NF-κ B is thought to be part of a stress response as it is activated by a variety of stimuli, including growth factors, cytokines, lymphokines, UV light, pharmacological agents, and stress.



Lane 1: control group; Lane 2: model group; Lane 3: therapy group. Values are presented as $\bar{x} \pm s$. **P* < 0.01 compared with the control group. #*P* < 0.01 compared with the model group.

Fig. 2 Detection of NF-κ B activity by EMSA

The various stimuli activate NF- κ B to cause phosphorylation of I κ B, followed by its ubiquitination and subsequent degradation^[7]. This results in the exposure of the nuclear localization signals(NLS) on NF- κ B subunits and the subsequent translocation of the molecule to the nucleus. In the nucleus, NF- κ B binds with a consensus sequence(5' GGGACTTCC-3') of various genes and thus activates their transcription. The NF- κ B system, consisting of NF- κ B/Rel protein and I κ B protein family, is involved in the control of infection, inflammatory reaction, oxidative stress, cell growth and apoptosis through regulating the expression of multiple genes. The genes regulated by NF- κ B include cytokines, chemokines, oxidation-associated enzymes and acute phase proteins^[4].

The activation of mesangial cells is a key step in the occurrence and development of renal diseases, where they act as both the participants and casualties. NF- κ B may play an important role in the glomerular immune complex reaction^[8]. During the course of disease progression, circulatory neutrophil activation is induced by immune-complex deposition, which leads to the production of a variety of cytokines, including TNF- α , IL-1 β and IFN- γ . This is followed by the activation of NF- κ B in mesangial cells, which is accompanied by cell proliferation and inflammatory factors release. In addition, it has been demonstrated that NF- κ B is also involved in the pathogenesis of chronic glomerulonephritis. Lee *et al*^[9] found that NF- κ B activation enhanced the macrophage infiltration in diabetic nephropathy; and over-activation of NF- κ B and AP-1 was reported in anti-Thy1.1-induced glomerulonephritis^[10].

Rhododendron Molle G. Don is a poisonous plant that is widely distributed in the South of China. Its flowers(nao yang hua) and fruits(ba li ma zi) are Chinese traditional medicines known for their effect as an analgesic, in lowering blood pressure, and for slowing heart rate. In addition they have uses as insecticides^[11]. However, because of their toxicity, they have to be used with caution for a short duration. It is only recently that there has been significant research on the pharmacological functions^[11,12] and the toxicity^[13] of roots of rhododendron. According to traditional medicine, preparations of rhododendron roots are pungent in flavor and warm in nature, and have the function of expelling wind and removing dampness, alleviating pain and swelling. Pro. Luo Yongyan^[14] has achieved some success in using roots of rhododendron to treat rheumatoid arthritis without any obvious side effect. Presently there is an increasing research focus on roots of rhododendron.

In this study, we have discussed the changes of NF- κ B expression, and the role of a rhododendron root preparation on NF- κ B, in chronic glomerulonephritis.

The immune complex glomerulonephritis model is characterized by the deposition of glomerular immune complex and the infiltration of macrophages. In our experiment, we added unilateral kidney removal and intraperitoneal administration of LPS to a traditional rat chronic glomerulonephritis model to accelerate the disease progression. The results show that roots of rhododendron can alleviate proteinuria and improve renal function. At the molecular level, a large amount of NF- κ B expression was found in the renal tissue of immune complex glomerulonephritis rats. Roots of rhododendron could have reduced the degradation of I κ B mRNA and the activation of NF- κ B, which would have inhibited the expression of downstream inflammatory factors and reduced the subsequent proliferation of cells and matrix. The results of the present study suggest that NF- κ B plays an important role in the pathogenesis of chronic glomerulonephritis and that roots of rhododendron might attenuate renal damage by downregulating the expression of NF- κ B in this model.

Studying immunosuppressor agents extracted from plants is popular in the field of pharmacology, because of the availability of plant material, the low price, and low toxicity of the resulting extracts. Researchers both in and out of China have achieved great success with such extracts. We have performed the preliminary study showing the therapeutic effect of roots of rhododendron on chronic glomerulonephritis. Rhododendron root extract may prove to be a highly effective, low cost and low toxicity clinical treatment of chronic glomerulonephritis.

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