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Research Article

Effect of Tripterine on Notch Signaling Pathway in IgA Nephropathy Rats

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Abstract: The objective of the study was to investigate the effect of tripterine on the Notch signaling pathway in renal tissue of IgA nephropathy rats. SD male rats were divided into the control group, IgA nephropathy model group, benazepril group, 1 mg/kg/day tripterine intervention group, and 10 mg/kg/day tripterine intervention group according to the random number table method, with 10 rats in each group. The urinary sediment and 24-h urinary protein quantity were detected by conventional methods. The expressions of Notch1, Jagged1, Hes1, and Hey1 in renal tissue of rats were detected by real-time fluorescent quantitative polymerase chain reaction. IgA nephropathy model was successfully established. The hematuria and proteinuria in model group were higher than those of control group ($P < 0.05$). The expressions of Notch1, Jagged1, Hes1, and hey1 in kidney tissue of IgA nephropathy rats were significantly increased ($P < 0.05$). Compared with the model group, hematuria and proteinuria in the tripterine intervention group were alleviated. The expressions of Notch1, Jagged1, Hes1, and Hey1 in rat renal tissue were decreased ($P < 0.05$). Moreover, the expressions of Notch1, Jagged1, Hes1, and Hey1 in renal tissue of rats in 10 mg/kg/day tripterine intervention group were decreased ($P < 0.05$). Tripterine can decrease the levels of hematuria and proteinuria in IgA nephropathy rats. The expression of the Notch signaling pathway in IgA nephropathy rats is increased by the down-regulation of tripterine, suggesting that tripterine has a certain therapeutic effect on IgA nephropathy rat. Moreover, its role may be realized through this signal pathway so as to provide a new mentality for the diagnosis and treatment of IgA nephropathy.

Keywords: *IgA nephropathy; Notch signaling pathway; tripterine; rats*

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0 Introduction

IgA nephropathy is the most common reason leading to end-stage renal disease (ESRD). IgA nephropathy accounts for 40–47.2% of primary glomerular disease in China. The report showed that the proportion was still showing an obvious uptrend[1,2]. Moreover, about 1.5% of them develops into ESRD after the initial symptom occurs in IgA nephropathy patients[3]. However, its pathogenesis is not clear. Clinical symptomatic treatment and immune inhibition are the main therapies. The Notch signaling pathway is a relatively conservative signal transduction pathway but widely existing in intercellular space. It is involved in the whole renal development process in mammals. In recent years, the research on the Notch signaling pathway is not only its effect in the renal development process. On the contrary, its effect in glomerular diseases is paid more attention. There are reports about its correlation with acute kidney injury, diabetic nephropathy, renal tubule interstitial fibrosis, renal tumor, and other diseases[4]. However, there was no correlated report about it in IgA nephropathy. The expression of Notch signal pathway in renal tissue was investigated in this experiment through the establishment of IgA nephropathy rat model so as to explore the approach and curative effect of tripterine.

1 Materials and methods

1.1 Materials

A total of 60 healthy male SD rats, weight about 150 g, were purchased from Beijing Weitong Lihua

Experimental Animal Technology Co., Ltd. The certificate number was SCXK (Beijing) 2012–0001. Bovine serum albumin (BSA) was purchased from American Sigma Company. Carbon tetrachloride (CCL4) was purchased from Tianjin Chemical Reagent Co. Ltd. Lipopolysaccharide (LPS) was purchased from American Sigma Company. Rabbit anti Notch1 polyclonal antibody was purchased from American Cell Signal Company. Rabbit anti Jagged1 polyclonal antibody and Rabbit anti-Hey1 polyclonal antibody were purchased from the American Santa Cruz company. Rabbit anti-Hes1 polyclonal antibody was purchased from American Abcam Company. Benazepri1 hydrochloride tab1et was provided by Beijing Novartis Pharma Ltd. Tripterine was purchased from Chengdu Pufeide Biological Technology Co. Ltd.

1.2 Establishment and grouping of IgA nephropathy animal model

A total of 60 male SD rats with SPF level were randomly divided into 14 rats in the control group and 46 rats in the model group. The rats in model group were given with 600 mg/kg BSA by gavage every other day. It was lasted for 12 weeks. 0.1 ml CCL4 + 0.5 ml castor oil was subcutaneously injected for 12 weeks, once a week. 0.05 mg/rat LPS was intravenously injected through caudal vein in 6 and 8 weeks to establish the model. The rats in the control group were given with isometric distilled water by gavage and isometric normal saline by subcutaneous injection. In the 13th week, 6 rats in the model group, and 4 rats in the control group were randomly selected to be executed. Their kidney tissue was detected by light microscopy and immunofluorescence to evaluate whether the modeling was successful. After successful modeling, the rats were randomly divided into the model group, benazepril group and 1 mg/kg/day tripterine intervention group, 10 mg/kg/day tripterine intervention group, with 10 rats in each group. Tripterine and benazepril were given from the 13th week to the end of the 20th week.

1.3 Sample collection

The rat urine was collected with a metabolic cage at the end of 12, 16, and 20th week for the detection of urinary sediment and urine protein. After the end of the experiment, the rats were anesthetized by 10% chloral hydrate. The abdomen was opened. The blood was collected from abdominal aorta for biochemical detection. The renal tissue was used for immunofluorescence, real-time quantitative polymerase chain reaction (PCR).

1.4 Immunofluorescence

The renal tissue of rats was removed, embedded with OCT and quickly frozen in liquid nitrogen. Then, the frozen sections were made into 3 μm sections. The frozen sections were dried, washed 3 times with phosphate-buffered saline (PBS) buffer, 3 min every time. Fluorescein isothiocyanate-labeled IgA antibody (1:50 dilution) was added, incubated in an incubator for 30 min at 37°C, washed for 3 times with PBS buffer, 3 min every time. The sections were sealed with glycerol and read under a fluorescence microscope. Immunofluorescence intensity classification semi-quantitative standards referred to five gradings commonly used at home and abroad: Not be displayed under low magnification, seemed invisible under high magnification ±; seemed invisible under low magnification and seemed visible under high magnification +; invisible under low magnification and clear under high magnification ++; clear under low magnification and dazzling under high magnification +++; and dazzling under high magnification ++++.

1.5 Real-time fluorescent quantitative PCR

The total RNA was extracted with the Total RNA Extraction Kit. The total volume of the reverse transcription reaction system was 20 μl. cDNA was synthesized according to the instructions of reverse transcription.

Primer sequences were:

Actb, upstream 5-CGTAAAGACCTC TATGCCAACA-3, downstream 5-GTTGGTGTCGCAGTTGGAG-3;

Notch1, upstream 5-GACCGTGTGG CTTCCTTCTA-3, downstream 5-GTTGGTGTCGCAGTTGGAG-3;

Jagged1, upstream 5-CATGGCCTC CAACGATACTC-3, downstream 5-GGTGAATTTGCCTCCTGACT-3;

Hes1, upstream 5-GTGGGTCCT AACGCAGTGTC-3, downstream 5-TGATTAGCAGTGGCCTGAGC-3;

Hey1, upstream 5-GGCTGAAGT TGCCCCTTAT-3; Downstream 5-GCTGGGATGCGTAGTTGTTG-3.

Table 1. Comparison of urine red blood cell count of rats at different time phases (x̄±S, / HP, *n*=10)

Time	Control group	Model group	Benazepril group	Low dose group ※	High-dose group ★
End of 12th week	0	12.4±3.42	10.3±3.06$^{\Delta\Delta}$	11.8±3.12$^{\Delta\Delta}$	10.7±3.09$^{\Delta\Delta}$
End of 16th week	0	14.7±3.16$^{\blacksquare}$	7.7±2.36$^{\square\Delta\#}$	9.9±3.18$^{\square\#}$	6.6±2.76$^{\square\#\Delta}$
End of 20th week	0	13.7±3.06	5.7±1.77$^{\blacktriangle\blacktriangledown\triangledown}$	5.9±1.45$^{\blacktriangle\blacktriangle}$	4.4±0.97$^{\blacktriangle\blacktriangledown}$

※ 1 mg/kg/day tripterine intervention group; ★ 10 mg/kg/day tripterine intervention group. Comparison between groups at the end of 12th week and 16th week, $^{\Delta\Delta}P$<0.05; compared with model group at the end of 16th week, $^{\square}P$<0.05; compared with low dose group at the end of 16th week, $^{\Delta}P$<0.05; compared with model group at the end of 20th week, $^{\blacksquare}P$>0.05, $^{\blacktriangle}$<0.01, $^{\blacktriangle\blacktriangle}P$<0.05; compared with low dose group at the end of 20th week, $^{\blacktriangledown}P$<0.05, compared with high dose group, $^{\triangledown}P$>0.05; comparison between all groups at the end of 16 weeks and 20 weeks, $^{\#}P$<0.05

Table 2. Comparison of 24-h urinary protein quantity of rats at different time phases (x̄±S, g/24 h, *n*=10)

Groups	End of 12th week	End of 16th week	End of 20th week
Control group	0	0	0
Model group	11.69±0.28$^{\Delta}$	11.74±0.71$^{\Delta}$	11.62±0.52$^{\Delta}$
Benazepril group	11.42±0.44$^{\Delta}$	7.22±0.28$^{\Delta\blacktriangle\square}$	6.55±0.33$^{\Delta\blacktriangle\square}$
Low-dose group ※	11.58±0.54$^{\Delta}$	9.05±0.44$^{\Delta\blacktriangle}$	8.22±0.30$^{\Delta\blacktriangle}$
High-dose group ★	11.59±0.67$^{\Delta}$	7.24±0.31$^{\Delta\blacktriangle\square\blacksquare}$	6.58±0.35$^{\Delta\blacktriangle\square\blacksquare}$

※ 1 mg/kg/dau tripterine intervention group; ★ 10 mg/kg/day tripterine group. Compared with the control group, $^{\Delta}P$<0.05; compared with the model group, $^{\blacktriangle}P$<0.05; compared with benazepril, $^{\blacksquare}P$>0.05; compared with 1 mg/kg/day tripterine intervention group, $^{\square}P$<0.05

Table 3. Comparison of blood biochemical indexes of rats in all groups (x̄±S, *n*=10)

Groups	BUN (mmol/L)	SCR (umol/L)	ALT (U/L)	AST (U/L)
Control group	8.51±0.37	30.68±1.71	54.06±1.43	123.93±9.21
Model group	8.38±0.21	31.51±2.50	55.30±1.92	127.41±8.71
Benazepril group	8.53±0.25	31.37±2.34	55.67±2.32	123.73±9.66
Low-dose group ※	8.64±0.27	30.01±2.17	53.42±3.01	124.97±10.23
High-dose group ★	8.47±0.32	30.77±2.01	55.45±3.07	126.45±8.44

※ 1 mg/kg/day tripterine intervention group; ★ 10 mg/kg/day tripterine intervention group, 2.3 Renal tissue immunofluorescence

They were synthesized by Shanghai Shenggong Biotechnology Engineering Company. The total volume of PCR reaction system was 20 μl, reaction conditions: Pre-degeneration at 95°C for 3 min; degeneration at 95°C for 12 s, annealing at 60°C for 40 s. There were 40 cycles. The experiment was repeated for 3 times for each sample and each gene. The gene copies of each sample were shown with Ct value. The relative expression of each gene was analyzed by 2- △△ Ct.

1.6 Statistical processing

All the collected data were processed by SPSS 17.0 statistical software. The measurement data were shown with the mean±standard deviation (x̄±S). The comparison between groups was shown with analysis of variance. The homogeneity of variance was shown with rank sum test. $P < 0.05$ showed the difference was statistically significant.

2 Results

2.1 General indexes

Compared with the control group, hematuria and 24-h urinary protein quantity in IgA nephropathy group were significantly higher ($P < 0.05$); compared with the model group, hematuria and 24-h urinary protein quantity in benazepril group and tripterine intervention group were decreased significantly ($P < 0.05$). The hematuria and 24-h urinary protein quantity in benazepril group and 10 mg/kg/day tripterine intervention group were decreased significantly than those of 1 mg/kg/day tripterine intervention group ($P < 0.05$) [Tables 1 and 2].

2.2 Analysis of blood biochemical indexes

Compared with the control group and model group, blood urea nitrogen, serum creatinine, alanine aminotransferase, and aspartate aminotransferase of

rats in the intervention group, the difference had no statistical significance ($P > 0.05$) [Table 3].

2.3 Renal tissue immunofluorescence

There was no mesangial IgA deposition in the control group. The renal tissue mesangial area of rats in the model group showed diffuse granular IgA deposition. The fluorescence intensity was ++ ~ +++, suggesting that the model was successful [Figure 1].

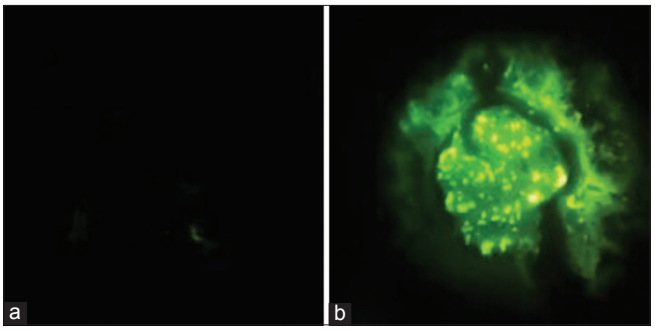

Figure 1. (*400) Renal tissue immunofluorescence results of rats (a) control group and (b) model group

2.4 Expressions of Notch1, Jagged1, Hes1, and Hey1 mRNA

Real-time fluorescence quantitative PCR results showed that compared with the control group, the levels of Notch1, Jagged1, Hes1, and Hes1 mRNA were increased in renal tissue of rats in model group ($P < 0.01$); the levels of Notch1, Jagged1, Hes1, and Hey1 mRNA in renal tissue of rats in benazepril group, 1 mg/kg/day tripterine intervention group, and 10 mg/kg/day tripterine intervention group were lower than those of the model group ($P < 0.05$), but still higher than those of the control group. Among them, there was no significant difference between the levels of Notch1, Jagged1, Hes1, and Hey1 mRNA in renal tissue of rats in benazepril group and 10 mg/kg/day tripterine intervention group ($P > 0.05$). However, compared with 1 mg/kg/day intervention group, the levels of Notch1, Jagged1, Hes1, and Hey1 mRNA in kidney tissue of rat were decreased significantly ($P < 0.05$) [Figure 2].

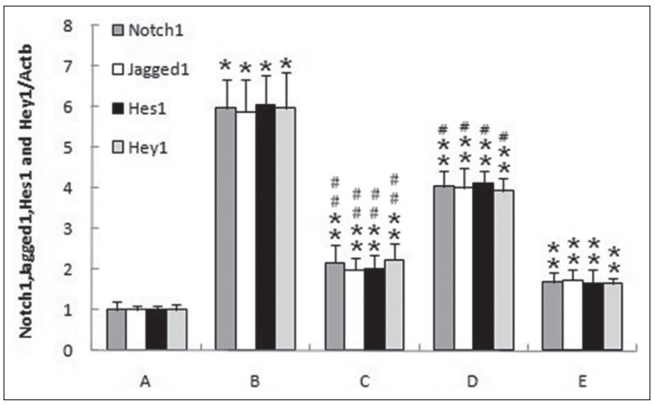

Figure 2. Expressions of Notch1, Jagged1, Hes1, and Hey1 mRNA in renal tissue of rats. A: Control group, B: Model group, C: Benazepril group, D: 1 mg/kg/day tripterine intervention group, E: 10 mg/kg/day tripterine intervention group. Compared with the control group, $*P < 0.01$. Compared with the model group, $**P < 0.05$. Compared with benazepril group and 10 mg/kg/day tripterine intervention group, $^{\#}P < 0.05$, compared with 10 mg/kg/day tripterine intervention group, $^{\#\#}P > 0.05$

3 Discussion

As the main reason for global glomerular disease and ESRD, IgA nephropathy plagued nephrologists and researchers for the long term. To understand the laws of occurrence and development, hereby as the basis to formulate scientific prevention and treatment measures, can better control the occurrence and development of disease, avoid or delay patients into ESRD stage, which has the realistic significance to the patient himself, country, and society.

Notch signaling pathway is a highly conserved signaling pathway widely existing in no spinal animal and spinal animals. There is the expression of the Notch signal pathway in three kidney development stages including pronephros, mesonephros, and metanephros, indicating its importance in kidney development. At present, the role of the Notch signaling pathway in renal disease has also been widely concerned. The combination of different receptors with the corresponding ligand was involved in the occurrence and development of different kidney diseases through activating Notch signaling pathway[5, 6].

This experiment showed that the expressions of important members Jagged1 and Notch1 in the Notch signaling pathway in renal tissue of IgA nephropathy rats were increased than those of the control group. The classical activated pathway of the Notch signaling pathway is also known as CBF-1/RBP-Jk dependent pathway. When the overexpressed Notch1 receptor was combined with its ligand Jagged1, released the activated form of Notch protein Notch intracellular domain (NICD) after pyrolysis for 3 times. NICD entered into the nucleus, combined CSL protein through RAM domain and cdc/ankyrin repetitive sequence, raised nuclear transcription, activated protein family MAML, formed transcriptional activator, post-transcribed and encoded basic helix-loop-helix family transcription factors including HES, HEY, and other target genes.

These transcription factors promoted the downstream gene expression[7]. The combination of Notch1 receptor with its ligand Jagged1 overexpressed in kidney tissue of IgA nephropathy rats activated the downstream target genes, resulting in the over-expression of Hes1 and Hey1. This showed that the Notch signaling pathway was activated in the pathogenesis of IgA nephropathy. The results provided new information for clinical research and treatment. We can slow down the progression of the disease by blocking or inhibiting the Notch signaling pathway.

Tripterine is a kind of active ingredient of traditional Chinese medicine Tripterygium Wilfordii. It has anti-inflammatory and immunoregulation effects. It not only has a unique curative effect on rheumatoid arthritis, systemic lupus erythematosus, and cancer but also plays a certain role in kidney disease. It can alleviate renal lesion and decrease proteinuria, but its therapeutic mechanism is not clear. This experiment showed that the activated Notch signaling pathway was down-regulated by the establishment of IgA nephropathy rat model, suggesting that the effect of tripterine in the treatment of IgA nephropathy was related with the Notch signaling pathway. Tripterine can inhibit the activation of the Notch signaling pathway, reduce proteinuria, alleviate glomerular sclerosis, and renal interstitial fibrosis, so as to reduce renal lesion and play a role in the treatment of disease. Moreover, the effect of the high dose group was more obvious than that of the low dose group. This experiment proved that Tripterine could treat IgA nephropathy rats by regulating the expression of the Notch signal pathway, which could provide a reliable experimental basis in treating IgA nephropathy and had certain promotional value and application prospect.

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