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Rhein Improves Sepsis Rats by Reducing Inflammatory Reaction and Regulating AQP2 Acute Renal Injury

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Abstract: Background: Inflammation is a typical manifestation of sepsis related acute renal injury. AQP2 is considered to play an important role in renal fluid transport. This study aims to explore whether rhein has a protective effect on renal injury and its potential mechanism. Methods: A sepsis rat model of acute renal injury was established. 60 SD rats were randomly divided into control group, model group, high rhein (200mg/kg), medium rhein (100mg/kg), low dose rhein (50mg/kg) and dexamethasone group. Observe the general condition of rats and detect serum creatinine (Scr), blood urea nitrogen (BUN) and TNF – α, IL-1 β, IL-10 expression; H&E staining was used to observe the pathological morphology of rat kidney; Immunofluorescence method was used to detect the expression of AQP2 in renal tissue: Western Blot method was used to detect the changes of protein expression of AQP2, NF-KB, P-NF-KB, IKB-α,P- IKB-α in renal tissue. Results: Compared with the control group, the levels of serum Scr, BUN and TNF- α, IL-1 β,and the level of IL-10 was significantly increased. There were obvious swelling of renal tubules, necrosis and abscission of epithelial cells of renal tubules, edema of renal interstitium, infiltration of inflammatory cells and other pathological damages in renal tissues; Compared with the model group, the rat serum Scr, BUN levels, TNF- α, IL-1β and the level of IL-10 was significantly reduced, and the pathological damage of renal tissue was significantly reduced. Conclusion: Rhein can reduce the inflammatory reaction, increase the expression of AQP2, and improve the acute renal injury in septic rats.

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1. Introduction

Acute kidney injury (AKI) is a disease that rapidly deteriorates renal function and even endangers life,[1]. About one third of sepsis patients will have AKI, and the annual incidence rate of sepsis related acute kidney injury (S-AKI) worldwide may be about 6 million cases or nearly 1 case per 1000 people [2]. The pathophysiological mechanism of S-AKI is very complex and has not been fully clarified. More and more evidence shows that it involves the interaction between inflammation, renal tubular epithelial cell damage and endothelial cell damage [3]. Lipopolysaccharide (LPS) is the main component of gram-negative bacteria and participates in the pathogenesis of endotoxemia induced AKI [4].

Rhein is a free anthraquinone compound separated from rhubarb. In recent decades, rhein has been widely used in the treatment of sepsis and other critical and critical fields [5]. Studies have found that [6, 7], rhein can down regulate proinflammatory cytokines and signal transduction through NF-KB and other signal pathways. Previous experiments have established that Rhein is effective in inhibiting fibronectin and the excessive inhibition of TGF-H, thereby reducing the accumulation of extracellular matrix, protecting renal function, and improving renal histological changes, which suggests that rhein may also play a protective role in renal injury.

Renal tubule function is regulated by aquaporin (AQP) and sodium transporter. AQP2 is a membrane protein family. AQP2 exists in the apical and subapical vesicles of the plasma membrane of the lumen. It allows water to enter the cell and plays a key role in regulating water balance. A previous study reported that the decrease of AQP expression was related to the damage of renal tubular epithelial cells [8]. On the contrary, the increase of AQP expression can improve the water metabolism in the kidney, thereby reducing the damage [9]. In addition, some studies have shown that endotoxemia can reduce the expression of AQP2, leading to the reduction of urine osmotic pressure. [10]

Therefore, the purpose of this article is to explore whether rhein can improve acute renal injury in septic rats by inhibiting inflammatory reaction and affecting the expression of AQP2 protein.

2. Materials and Methods

2.1. Animals

60 SPF male SD rats, weighing 300-400g, were purchased from the Experimental Animal Center of the Air Force Military Medical University. All animals adapt to the temperature (22± 2℃), humidity (50-60%) and light conditions (12h light/dark cycle) for at least 1 week.SD rats were intraperitoneally injected with lipopolysaccharide (LPS) at a dose of 10mg/kg to establish sepsis rat model. The rats were randomly divided into 6 groups (n=10 in each group): (1) control group, (2) model group, (3) high dose rhein group (200mg/kg, Rhein-H), (4) medium dose group (100mg/kg, Rhein-M), (5) low dose group (50mg/kg, Rhein-L), (6) dexamethasone group (Dex).

2.2. Assessment of Biochemical Parameters.

After pentobarbital anesthesia, blood samples were collected and centrifuged at 4℃ for 15 min after standing. Detect the Scr and BUN levels with automatic biochemical analyzer

2.3. Histological Analysis

After the rats were killed, renal tissue was collected, fixed with 4% paraformaldehyde solution. After 70% ~ 100% gradient ethanol solution dehydration, xylene transparency and paraffin

embedding, the slicer was used to make 2μm pathological sections. Then stained with hematoxylin eosin (HE).The morphology of kidneys in each group was observed under conventional light microscope. Three to five pieces were selected from each field of vision and photographed for preservation.

2.4. Analysis of Inflammatory Factors in Kidney Tissues

TNF-α (No.88-7340),IL-10(No.88-50629) and IL-1β(No.EK301B/3-02) were estimated using ThermoFisher Scientific ELISA kit to measure according to the instructions.

2.5. Immunofluorescence

Fixed with 4% paraformaldehyde for 20 min; 0.5% Triton X-100 is transparent for 15 min; 1% BSA for 30 min blocking, primary antibody was added and refrigerator at 4℃ overnight. Add fluorescent secondary antibody and incubate at room temperature for 1 h. DAPI staining for 20 min at room temperature; Cover sheet; Observe and take photos under fluorescence microscope.

2.6. Western Blot

Kidney tissue is fully mixed with RIPA lysate and cracked on ice, then centrifuge to extract the supernatant. Use BCA protein quantitative kit to quantify each protein sample, and add a proper volume of protein loading buffer to set the protein concentration at 10μg/μL. The sample solution is loaded for electrophoresis, and the protein is separated and transferred to the nitrocellulose membrane. Prepare 5% skimmed milk with TBST solution containing 0.1% Tween-20, place the nitrocellulose membrane in skimmed milk, seal it at room temperature for 2 hours, and incubate the first antibody at 4℃ overnight. Then incubate the second antibody for 1 hour, wash the membrane, and detect the strip with ECL luminous solution. The strip was imaged with Bio Rad gel imaging system, and the gray value was analyzed with the matching Image J software.

2.7. Statistical Analysis

The statistical software SPSS 26 was used to conduct statistical analysis on the experimental data. The measurement data were represented by mean±S.D. $P<0.05$ indicates that the difference is statistically significant, and $P<0.01$ indicates that the difference is extremely significant.

3. Results

3.1. Effect of Rhein on Renal Function in Rats

In order to explore the protective effect of rhubarb on renal injury in sepsis, rats were given rhubarb after LPS, and renal function indexes were measured at the end of the experimental period. Compared with the control group, the levels of BUN and Scr in the model group were significantly higher after LPS injection. However, rhubarb treatment effectively reduced the increase of these biochemical parameters. Compared with the model group, the levels of BUN and Scr in rats in the high, middle and low dosage groups of rhein were significantly reduced in a dose dependent manner ($P<0.05$ or $P<0.01$). (Table 1)

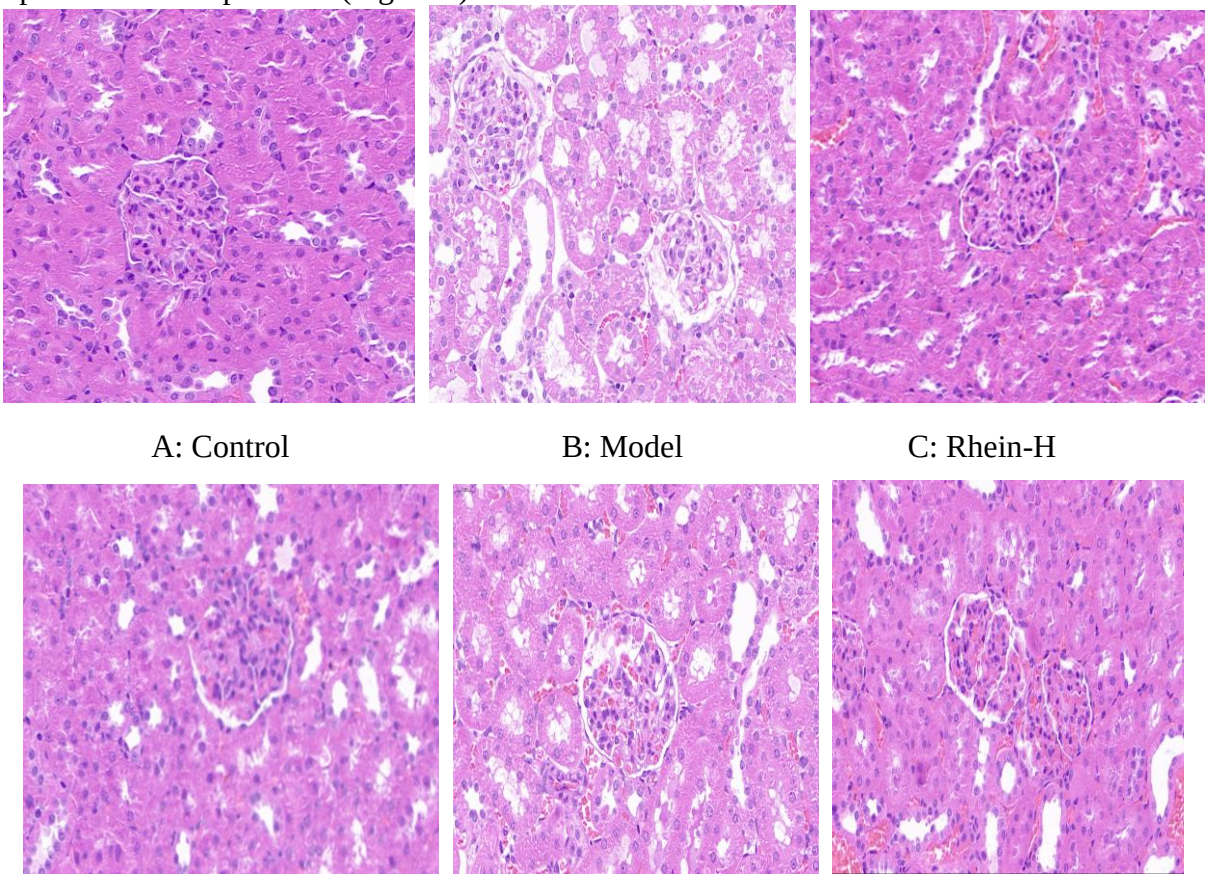
Table 1. Effect of rhein on renal function in rats

Group	Bun (mmol/l)	Scr (μmol/l)
ontrol	6.244±1.018	52.57±12.01
Model	33.94±4.336##	85.32±10.29##
Rhein-h	11.03±2.169**	54.90±7.590**
Rhein-m	19.75±5.001**	70.60±4.264**
Rhein-l	28.58±2.267**	82.79±5.844**
Dex	19.15±2.379**	51.48±11.46**

Compared with the control group, ##P < 0.01,#P < 0.05;Compared with the model group,**P < 0.01,*P < 0.05.

3.2. Rhein Can Alleviate Pathological Damage of Renal Tissue in Rats

The renal tissue of rats in the control group was clear and intact without damage. In the model group, glomerular mesangial hyperplasia, interstitial congestion and edema, balloon adhesions, inflammatory cell infiltration and other pathological damage were observed in the renal tissue of rats. Compared with the model group, the pathological damage of renal tissue of rats in the high, medium and low dose groups of rhein and dexamethasone groups were alleviated, and the rhein groups were dose dependent. (Figure 1)



A: Control B: Model C: Rhein-H

D: Rhein-M E: Rhein-L F: Dex

Figure 1. H&E staining for kidney sections(×400)

3.3. Rhein Can Reduce the Increased Levels of Inflammatory Factors in Rats

Compared with the control group, the model group rats' serum TNF- α , IL-10 and IL-1β levels increased significantly; Compared with the model group, the high, middle and low dose groups of rhein had significant decreases (P<0.05 or P<0.01). These results suggest that the protective effect of rhubarb on sepsis induced renal injury is partly due to its anti-inflammatory effect. (Table 2)

Table 2. Effect of rhein on the level of inflammatory factors in rats

Group	Tnf-α(pg/ml)	Il-10(pg/ml)	Il-1β(pg/ml)
Control	1.239±0.7050	10.79±2.906	31.36±9.822
Model	71.33±11.35##	65.22±7.950##	792.3±23.64##
Rhein-h	30.72±2.496**	25.53±5.678**	127.7±35.90**
Rhein-m	44.47±9.864**	42.34±2.199**	448.0±34.90**
Rhein-l	57.08±3.196**	51.06±3.783**	655.9±38.99**
Dex	43.21±8.471**	32.07±9.291**	389.6±13.21**

3.4. Inhibitory Effect of Rhein on Renal AQP2 Protein Expression in Septic Rats

To further investigate the effect of rhein on renal AQP2 in septic rats, the expression level and difference of renal AQP2 were observed. The results showed that AQP2 expression was decreased in the model group compared with the control group; however, the decrease in AQP2 expression was effectively reversed in the Rhein-H group. (Figure 2)

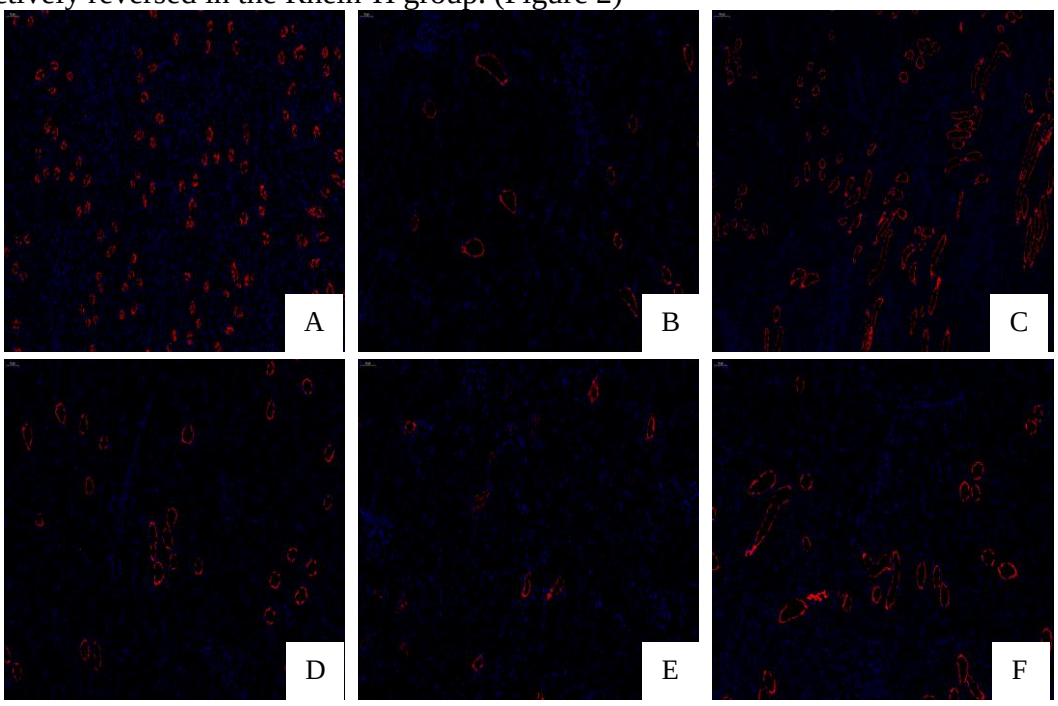


Figure 2. Immunofluorescence analysis of the effect of rhein on AQP2 expression in rats (×200) . A: Control B: Model C: Rhein-H D: Rhein-M E: Rhein-L F: Dex

In addition, Western blot results showed that AQP2 expression was significantly decreased in LPS treated model rats. However, these changes could be blocked by rhein treatment. (Figure 3)

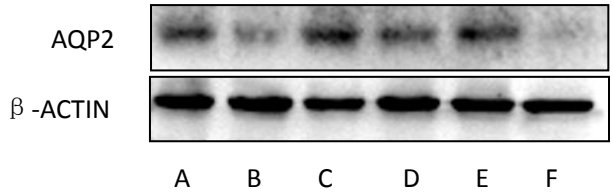


Figure 3. Effect of rhein on AQP2 protein expression in rat kidney.

3.5. Effect of Rhein on the Activation of the IKB-A /NF-KB Pathway.

At present, LPS induced inflammation is mainly caused by transcription level NF-KB and IKB-α Phosphorylation regulation. LPS Promotes IKB-α. And rhein has inhibitory effect on it. (Figure 4)These results suggest that rhein reduces inflammation by inhibiting the activation of IKB-α /NF-KB pathway.

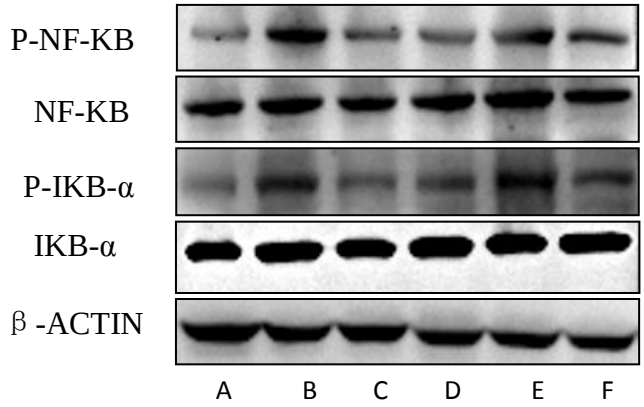


Figure 4. Effect of Rhein on the activation of the IKB-α /NF-KB pathway

4. Discussion

The sepsis rat model was established by intraperitoneal injection of LPS type. The results showed that inflammatory cells increased glomerular mesangial hyperplasia, interstitial congestion and edema, and other pathological damage in the model group. Rat serum Scr, BUN TNF - α ,IL-10 and IL-1β elevated levels. It shows that LPS can cause severe inflammatory reaction in rats, damage renal tissue, abnormally increase renal function indicators, and cause renal dysfunction, which reveals that the model is successfully established.

In this study, we provided evidence that rhein improved renal function in septic AKI rats, was associated with histopathological changes, and reduced renal inflammation. In addition, rhubarb could inhibit LPS induced decrease of aquaporin expression.

AQP2, also known as aquaporin, is a complete membrane protein that comes from a large family of major intrinsic proteins, forms the membrane pores of biological cells, and promotes intercellular water transport and intracellular turnover [11]. It is an important part of liquid transportation. Its dysfunction leads to the imbalance of liquid transportation and pathological changes. AQP2 is critical to the permeability of watercourses under high permeability gradient. Previous studies have shown that AQP2 may play a key role in trans membrane water transport and liquid reabsorption, and may be closely related to kidney damage [12-14]. Through experiments, we found that Rhein increased AQP2 protein expression and reduced water reabsorption in the model group.

As a transcription factor, NF-kB is the convergence point of many signal transduction pathways, plays a key role in regulating inflammatory response, and is also an proinflammatory cytokine activate one of the main signal pathways of cells. When the cell is at rest, NF-KB forms a trimer with the inhibitor protein (Ik B), so NF-KB exists in the cytoplasm in an inactive form. When cells are stimulated by external signals, IKB phosphorylates and degrades. At this time, NF-KB is activated without the strict control of IKB, enters the nucleus, combines with the corresponding part of the target gene, and starts the transcription of related genes [15, 16].

The above studies showed that Rhein inhibited the inflammatory response, increased AQP2 content, reduced renal tissue damage, and ultimately prompted the recovery of renal function.

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Data Availability

The datasets used during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The author states that this article has no conflict of interest.

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