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Gallic acid alleviates lipopolysaccharide-induced renal injury in rats by inhibiting cell pro-death and inflammatory response and its mechanism

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ABSTRACT

The mechanism of gallic acid in improving lipopolysaccharide-induced renal injury in rats was investigated by studying the pro-death and inflammatory response of cells. SPF rats were randomly divided into 4 groups with n=10 in each group. Blank control group: normal saline injection; The model group was injected with LPS induced model (LPS group); Low dose gallic acid group (LPS+L-GA group); Middle dose gallic acid group (LPS+M-GA group). The expression of serum inflammatory factors IL-1, IL-1β, IL-18, and MCP-1 were detected by Elisa. Western blot assay was used to detect the expression of inflammation-related proteins. The contents of BUN, Scr, SUA, Serum cystatinALB, and ACR were determined by the biochemical analyzer. The pathological tissue sections were used to observe the kidney injury in each group. The renal expressions of NLRP3, Caspase-1, GSDMD, and IL-1β were detected by immunohistochemistry. The activation of the AMPK/SIRT1 signaling pathway was detected by Western blot assay. The LPS-induced mouse kidney injury model was established successfully. Compared with the model group, different doses of gallic acid can improve the expression of renal biochemical indexes (P<0.05); At the same time, gallic acid can activate AMPK/SIRT1 and reduce kidney injury in mice (P<0.05); Compared with the model group, the expression of pyroptosis gene, the expression of genes related to inflammatory factors and the expression of inflammatory factors were decreased in the gallic acid injection group (P<0.05). By activating the AMPK/SIRT1 signaling pathway, gallic acid can inhibit the scorch death and validation effect in mice, thereby protecting the kidneys of mice.

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Introduction

Physical trauma can affect anyone and is responsible for more than 1 in 10 deaths worldwide. Although direct severe kidney injury is relatively rare, extrarenal tissue damage often leads to the development of acute kidney injury. Traumatic AKI (TRAKI) can be triggered and aggravated by a variety of causes, including hemorrhagic shock, rhabdomyolysis, nephrotoxic drug use, and infectious complications, especially in the presence of pre-existing or around-specific risk factors (1-3). The damage of kidney disease is often accompanied by inflammatory effects. Pyroptosis is a gastrin-mediated form of programmed cell death in which cells continue to expand until the cell membrane bursts, resulting in the release of cell contents that activate intense inflammatory and immune responses. Gallic acid is a natural polyphenolic compound that is widely found in Chinese herbs and fruit plants, including extracts of phyllophyllum, gallnut, tangerine, grape, sweet tea, primulin leaf, etc. Gallic acid can inhibit the growth of some tumors in vivo and in vitro, such as human esophageal cancer (4) and liver cancer (5). CAI Long's research confirmed that gallic acid can inhibit inflammatory effects in mammals (6).

At present, there are few studies on the effect of gallic acid on nephropathy, but according to relevant literature reports, gallic acid can inhibit the validation effect, so we try to study the animal model of nephropathy, hoping to provide new insights and basis for the development of drugs to treat nephropathy.

Materials and Methods

Experimental animals: SPF rats, 30 healthy male Wist ar rats aged 3-4 months, weighing 200-240 g, purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). This study was approved by the Animal Ethics Committee of the Shanghai University of Traditional Chinese Medicine Animal Center.

The drug and reagent: Gallic acid (GA) were purchased from Sigma Company (St. Louis, MO, USA). E. coli endotoxin (LPS) chemical pure (Sigma Corporation, St. Louis, MO, USA)); IL-1, IL-1β, IL-18, MCP-1 detection kit, Wuhan Fenn Biotechnology Co., LTD. (Wuhan, China). Rabbit anti-NLRP3, Caspase-1, GSDMD, and IL-1β were purchased from Cell Signaling Technology Co., LTD. (Danvers, MA, USA). AMPK/SIRT1 signaling pathway-related antibodies were purchased from Wuhan

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Sanying Biotechnology Co., LTD. (Wuhan, China).

Instrument TBA-120FR automatic biochemical analysis instrument, Toshiba Corporation of Japan; HBS-1101 enzyme label instrument, products of Nanjing De Tie Experimental Equipment Co., LTD. (Nanjing, China); 37XE-PC microscope, products of Shanghai Optical Instrument Factory 1 (Shanghai, China).

Methods

Experimental grouping

Grouping and handling measures After 1 week of adaptive feeding, the animals were randomly divided into 4 groups: healthy control group (n=10), 1 mL of normal saline was injected into the tail vein; In the LPS model group (n =10), the dose of LPS was 5 mg/kg body weight, and normal saline was diluted to 1 ml tail vein injection, resulting in AKI model. The low-dose GA intervention group (n=10), that is, the LPS+L-GA group, was given the same standard as the LPS group, while the gallic acid was given 50 mg/kg by gavage of low and high doses of gallic acid, respectively. Low-dose GA intervention group (n=10), that is, LPS+H-GA group 100 mg/kg body weight, diluted with normal saline to 1 mL tail vein injection. After drug administration, rats were placed in a metabolic cage to collect urine and observe and compare the breathing, spirit, appetite, urine color, and other general conditions of animals in each group. After 6 h, the experimental animals were anesthetized by intraperitoneal injection of 25% elastane (1.2 g/kg body weight), and the marks were collected.

Observation indicators

Western blot

The rat kidney tissue was extracted, the protein cracking reagent radioimmunoprecipitation assay (RIPA): phenylmethylsulfonyl fluoride (PMSF)=99:1 was added, the protein cracking reagent was fully cracked, and the supernatant was absorbed after 12000 g centrifugation for 10 minutes. The protein sample was mixed with the sample buffer at a ratio of 4:1 and combined in a water bath at 100°C for 15 minutes. The sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel was prepared by conventional method, the sample was added in the electrophoresis hole, and the 75 V voltage was electrophoreted in the concentrated gel and the 120 V voltage was electrophoreted in the separation gel. 200 mA constant flow membrane 30 min. Polyvinylidene fluoride (PVDF) membrane was placed in 5% skim milk powder and combined at room temperature for 1 h. PVDF membrane was placed in the primary antibody and reacted overnight at 4°C. Finally, the PVDF membrane was placed in the secondary antibody and combined at room temperature for 30 minutes. NLRP3, Caspase-1 and IL-1β, p-AMPK, AMPK, and SIRT1 protein were diluted at 1:1000 for the primary antibody, 1:8000 for the primary antibody, and 1:3000 for the secondary antibody of GSDMD. ECL luminescence, Image J analysis of gray values of bands, GAPDH as a reference to analyze the change of target protein expression.

Automatic biochemical analyzer to detect BUN, Scr, SUA, Serum cystatinALB, ACR content

Serum levels of BUN, Scr, SUA, Serum cystatinALB, and ACR were detected by an automatic biochemical analyzer.

Immunohistochemical test and pathological film of kidney tissue

(1) Dissociated right kidney. Soaked in 10% neutral formaldehyde solution for 24 h, dehydrated, embedded, paraffin sections, dewaxed in xylene, dehydrated with gradient alcohol, incubated with 3% hydrogen peroxide for 15 min to block endogenous peroxidase, washed with distilled water for 2 min, Tween washed for 5 min×3 times. Microwave antigen repair for 13 min, T-ween washing for 5 min ×3 times, adding 1:200 mouse monoclonal NLRP3, Caspase-1, GSDMD, and IL-1β antibodies at 4°C overnight, and placing them at room temperature for 20 min after removal from the refrigerator. Polymer Helper was incubated at 37°C for 20 min and washed the same as above. poly peroxidase-anti-mouse/rabbit IgG was incubated at 37°C for 30 min and washed the same as above. Finally, DAB color developer was added for 10 min, the reaction was terminated with tap water, hematoxylin restained the nucleus, and the results were observed after dehydration, transparency, and tablet sealing. Result observation: A section with clear staining was taken from each kidney tissue specimen, and each section was randomly selected with 3 fields of view under a light microscope (400 times). With a fixed window area, the BI-2000 image analysis system (Chengdu Taiming Technology Co., LTD., Chengdu, China) was used to determine the positive area ratio. The brown-yellow particles protruding from the background in the cell were positive, and no brown-yellow deposition or light coloring was found, which was almost the same as the background. The larger the positive area, the more protein content, and the stronger the expression. (2) HE staining was performed to observe the renal tissue injury.

ELISA was used to detect TNF-α and IL-6

The rat kidney tissues were collected and the contents of IL-1, IL-1β, IL-18, and MCP-1 were determined by ELISA. For specific operation methods, refer to the kit instructions.

Statistical Analysis

Statistic Package for Social Science (SPSS) 25.0 statistical software (IBM, Armonk, NY, USA) was used for statistical analysis, and the measurement data were expressed as $\bar{x}\pm s$. ANOVA was used for the comparison of multiple groups, followed by a homogeneity test of variance and a homogeneity test of variance. q test was used for poundwise comparison among all groups. $P<0.05$ indicated that the difference was statistically significant.

Results

Gallic acid can improve the pathological changes of renal tissue in rats with renal injury

H&E staining results of the renal cortex (Figure 1) showed that compared with the control group, most of the model group showed inflammation, the number of renal tubules decreased, renal structure atrophy disappeared, and interstitial lymphocyte infiltration was more. Some of the surrounding renal tubules were enlarged and the epithelial cells were edema. The renal tissue was improved significantly in the low-dose and medium-dose groups, and

the edema of renal tubular epithelial cells was weakened. The lumen diameter of the renal tubule decreased. These results indicated that different doses of gallic acid could improve the LPS-induced injury model in rats.

Effects of gallic acid on the expression of pyroptosis-related proteins in rat renal tissue

The results of Western blot and immunohistochemical experiments confirmed that compared with the model group, the expressions of NLRP3, Caspase-1, GSDMD, and IL-1β in renal tissues of rats in different gallic acid groups were decreased (P<0.05), as shown in Figure 2 and Table 1. These results indicated that different doses of gallic acid could decrease the expression of pyrogenic protein in the renal tissue of rats.

Gallic acid can improve renal function in rats with renal injury

The contents of blood urea nitrogen (BUN), serum creatinine (Scr), serum cystatin (cystatin), serum uric acid (SUA)/ serum albumin (ALB), and urinary albumin/creatinine ratio (ACR) were measured by biochemical method. As shown in Table 2, compared with the model group, the renal function indexes of the gallic acid group were improved (P<0.05), indicating that gallic acid can improve the kidney injury induced by LPS in rats.

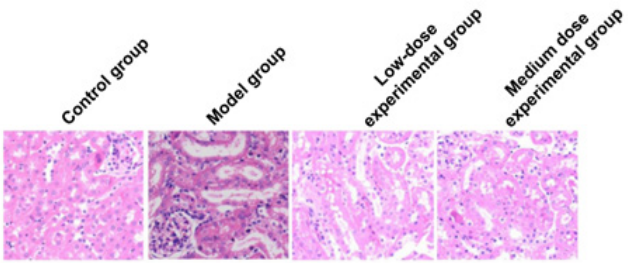

Figure 1. Histopathological changes of kidney sections in each group.

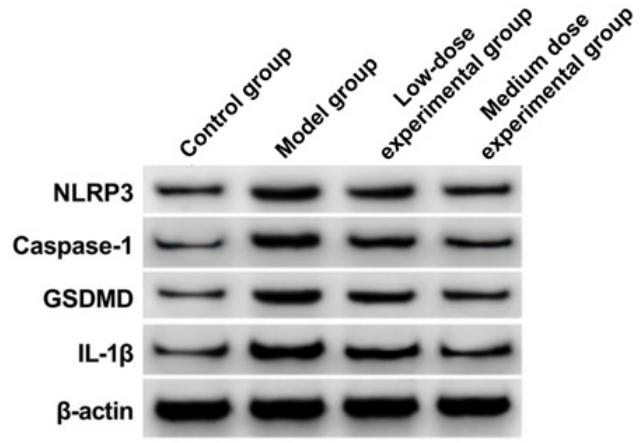

Figure 2. Expression of NLRP3, Caspase-1, GSDMD and IL-1β in each group.

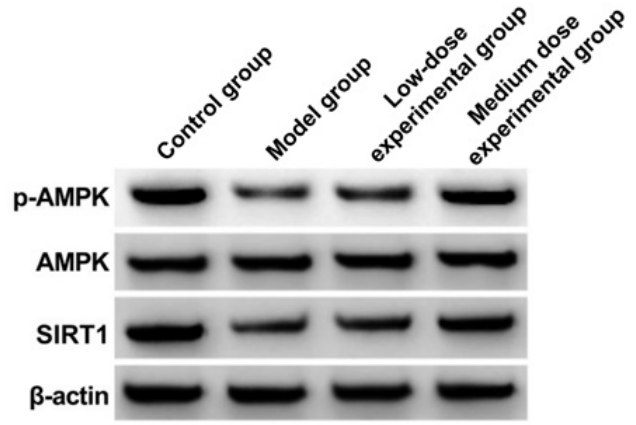

Figure 3. Comparison of protein expression levels of p-AMPK, AMPK, and SIRT1 in renal tissues of rats in each group.

Gallic acid alleviates inflammation in rats with renal injury

The expression of inflammatory factors can reflect the inflammation-related situation of organisms. In this study, ELISA was used to detect the changes in serum levels of IL-1, IL-1β, IL-18, and MCP-1 of rats in each group under the action of gallic acid, as shown in Table 3. Compared with the model rats, The expression level of serum inflammatory factors in the gallic acid group was significantly decreased (P<0.05), indicating that gallic acid could improve the expression of serum inflammatory factors induced by LPS.

Gallic acid activates AMPK/SIRT1 signaling pathway

AMPK/SIRT1 signaling pathway can protect the mammalian neural network, and reduce the transcription of inflammation-related factors in the body, and its activation can reduce the inflammatory effect of the body to a certain extent. We detected the expression of P-AMPK, AMPK, and SIRT1 proteins in the kidney tissues of rats in each group through Western blot assay. It was found that, compared with rats in the model group, different amounts of gallic acid could activate the expression of the AMPK/SIRT1 signaling pathway (P<0.05), as shown in Figure 3 and Table 4. This means that different amounts of gallic acid can improve the inflammatory effect of rats at the molecular level.

Discussion

Relevant studies have reported that Acute respiratory distress syndrome (ARDS) and AKI are the most common injuries to organs, which are mainly due to the uncontrol-

Table 1. Comparison of NLRP3, Caspase-1, GSDMD and IL-1β protein levels in each group ($\bar{x}$±s, n=10).

Group	NLRP3	Caspase-1	GSDMD	IL-1β
Control group	0.29±0.06	0.20±0.06	0.19±0.03	0.20±0.05
Model group	0.88±0.10*	1.01±0.10*	0.86±0.07*	0.96±0.11*
Low-dose experimental group	0.64±0.07Δ	0.68±0.07Δ	0.56±0.08Δ	0.60±0.10Δ
Medium dose experimental group	0.43±0.06Δ▲	0.52±0.05Δ▲	0.45±0.04Δ▲	0.36±0.05Δ▲

Compared with control group, *P<0.05; Compared with the model group, ΔP<0.05; Compared with low-dose experimental group, ▲P<0.05.

Table 2. Comparison of renal function indexes of rats in all groups ($\bar{x}$±s, n=10).

Group	BUN (mmol/L)	Scr (μmol/L)	SUA (μmol/L)	Serum cystatin(mg/L)	ALB (g/L)	ACR (mg/g)
Control group	3.93±0.43	28.65±4.12	100.00±10.47	1.02±0.14	33.61±4.42	0.15±0.004
Model group	9.39±1.15*	67.18±5.63*	164.90±20.98*	1.65±0.14*	24.61±1.45*	0.19±0.007*
Low-dose experimental group	6.67±0.42Δ	54.39±5.51Δ	144.58±8.00Δ	1.46±0.15Δ	29.24±2.14Δ	0.18±0.004Δ
Medium dose experimental group	4.76±0.34Δ▲	41.13±4.30Δ▲	126.61±16.34Δ▲	1.27±0.10Δ▲	32.33±1.63Δ▲	0.17±0.004Δ▲

Compared with control group, *P<0.05; Compared with the model group, ΔP<0.05; Compared with low-dose experimental group, ▲P<0.05.

Table 3. Comparison of serum IL-1, IL-1β, IL-18 and MCP-1 levels of rats in each group ($\bar{x}$±s, n=10).

Group	IL-1(pg/mL)	IL-1β(pg/mL)	IL-18(pg/mL)	MCP-1(pg/mL)
Control group	22.16±2.29	9.73±1.66	57.96±1.82	22.14±2.06
Model group	56.15±4.03*	32.93±2.20*	135.25±21.01*	89.60±8.62*
Low-dose experimental group	43.44±6.09Δ	23.00±1.54Δ	102.69±9.47Δ	64.86±9.34Δ
Medium dose experimental group	31.13±3.41Δ▲	15.53±1.87Δ▲	78.64±7.75Δ▲	41.14±3.42Δ▲

Compared with control group, *P<0.05; Compared with the model group, ΔP<0.05; Compared with low-dose experimental group, ▲P<0.05.

Table 4. Comparison of protein expression levels of p-AMPK, AMPK, and SIRT1 in renal tissues of rats in each group ($\bar{x}$±s, n=10).

Group	p-AMPK	AMPK	SIRT1
Control group	0.89±0.11	0.82±0.10	0.94±0.15
Model group	0.22±0.05*	0.79±0.13*	0.36±0.05*
Low-dose experimental group	0.38±0.04Δ	0.80±0.13Δ	0.58±0.10Δ
Medium dose experimental group	0.68±0.09Δ▲	0.78±0.12Δ▲	0.84±0.11Δ▲

Compared with control group, *P<0.05; Compared with the model group, ΔP<0.05; Compared with low-dose experimental group, ▲P<0.05.

led biological inflammatory response and the participation of various inflammation-related factors (7-9). In recent decades, although China's nephrology and critical care medicine have made remarkable progress, the incidence and mortality of AKI are still high, which has become one of the major problems in critical care emergency medicine (10,11). Attention should be paid to the early diagnosis and treatment of kidney injury because studies have shown that most kidney damage has occurred before some biochemical indicators of evidence.

Gallic acid, chemical name is 3,4, 5-trihydroxybenzoic acid, molecular formula C7H6O5, is a kind of polyphenolic organic compound, that widely exists in palmatophyllum rhubarb, eucalyptus, dogwood, and other plants, and has a wide range of applications in food, biology, medicine, chemical and other fields. Relevant studies have confirmed that it has a certain effect on inhibiting the proliferation of cancer cells, and at the same time, it may have a certain effect on inhibiting the inflammation of mammalian cells (12,13). As for the biological marker for early diagnosis of AKI, the newly discovered IL-18 has received much attention in recent years. IL-18 is a widely distributed cytokine that was originally named interferon-gamma (IFN-γ) inducer. It is the key factor of endotoxin-induced MODS in rats. There is a certain amount of IL-18 in the normal human body, which can improve the body's immunity, but excessive expression will cause damage to the body and aggravate inflammation. Studies have shown that the expression of IL-18 in blood and urine increases significantly during acute tubular necrosis (ATN). In addition, other inflammation-related factors can also be used as relevant indicators to evaluate kidney disease to a certain extent (14,15). AMPK/SIRT1 plays an important role in the inflammatory process by regulating the gene expression of many important cytokines, adhesion molecules, and chemokines, which can participate in various immune and inflammatory reactions in the body. LPS can induce the production of various cytokines through NF-κB, and thus participate in the pathological process of endotoxic kidney injury. AMPK/SIRT1 has been shown to protect nerve cells from damage (16,17) and also reduce the inflammatory effect of cells (18). In this study, IL-18 and other inflammation-related factors, AMPK/SIRT1 signaling pathway, Ccr and other kidney-related indicators, and renal histopathological changes during AKI, as well as the effects of GA on these inflammatory factors and renal function indicators were compared to understand whether GA has a protective effect on the kidney during endotoxin-induced AKI in rats. To provide a new treatment plan for clinical treatment of AKI.

This study confirmed that both low and high doses of gallic acid can activate the AMPK/SIRT1 signaling pathway and improve the inflammatory effect in rat models. Improvement of inflammatory effect. We used the Elisa test to detect serum inflammatory factors, and the subsequent pathological sections of mouse kidneys and immunohistochemical detection confirmed the improvement of the inflammatory effect. At the same time, it was

further confirmed at the molecular level. The results of the Western blot experiment showed that the expression of inflammation-related factors and the protein of pro-death also decreased in the gallic acid group, which confirmed the inhibitory effect of gallic acid on inflammation-related proteins at the protein level.

In summary, our results confirmed the effect of gallic acid on inflammation and the inhibition of pyroptosis in rat models of nephropathy, indicating that gallic acid can improve the inflammatory damage of mammalian kidneys. Our results provide a basis for the development of novel drugs and the selection of drug targets.

Conflict of Interests

The authors declared no conflict of interest.

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