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HEALTH SCIENCES

Huanglian Decoction treats Henoch-Schonlein purpura nephritis by inhibiting NF-κB/NLRP3 signaling pathway and reducing renal IgA deposition

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Abstract: Henoch-Schonlein purpura nephritis (HSPN) is a systemic vascular inflammatory disease. Huanglian Decoction (HLD) ameliorates renal injury in nephritis; however, the mechanism of action of HLD on HSPN has not been investigated. This study aimed to investigate the protective mechanism of HLD treatment in HSPN. The effects of HLD on HSPN biochemical indices, kidney injury and NF-κB/NLRP3 signaling pathway were analyzed by biochemical analysis, ELISA, HE and PAS staining, immunohistochemistry, immunofluorescence, and Western Blot. In addition, the effects of HLD on HSPN cells were analyzed. We found that HLD treatment significantly reduced renal tissue damage, decreased the levels of IL-17, IL-18, TNF-α, and IL-1β, and increased the levels of TP and ALB in HSPN mice. It also inhibited the deposition of IgA, IgG, and C3 in kidney tissues and significantly decreased the expression of IκBα, p-IκBα, NLRP3, caspase-1, and IL-1β in kidney tissues and cells. In addition, PMA treatment inhibited the above-mentioned effects of HLD. These results suggested that HLD attenuates renal injury, IgA deposition, and inflammation in HSPN mice and its mechanism of action may be related to the inhibition of the NF-κB/NLRP3 pathway.

Key words: Henoch-Schonlein purpura nephritis, Huanglian Decoction, NF-κB/NLRP3 signaling pathway, IgA deposition.

INTRODUCTION

Henoch-Schonlein purpura nephritis (HSPN) is a systemic vascular inflammatory disease, which is a disease of allergic purpura involving the kidneys, with clinical manifestations of hematuria, proteinuria, rash, abdominal pain, and arthralgia, and the incidence of HSPN has been on the rise in recent years (Jelusic et al. 2019, Reamy et al. 2020). HSPN can occur at any age, with prevalence under 10 years of age and more males than females (Kurt-Şükür et al. 2021). Kidney involvement occurs around 1-4 weeks after the rash appears (Zhang et al. 2021b). Although most patients can recover spontaneously, there are still some patients who have recurrent episodes and the disease is persistent. As the number of recurrent episodes increases, the kidney damage gradually worsens, and some patients progress to end-stage disease after several years (Liu et al. 2018). Modern medicine is still exploring the etiology and exact pathogenesis of HSPN, and hormone or hormone combined with immunosuppressant is the most commonly used treatment method. However, due to the side effects during the treatment, it does not effectively control the recurrence of purpura and does not improve the prognosis of HSPN (Nickavar & Sadeghian 2017). Studies

have reported that Traditional Chinese medicine (TCM) has unique advantages in boosting immunity and improving proteinuria (Xue et al. 2021). Therefore, it is especially important to play the role of TCM in the treatment of HSPN.

Huanglian Decoction (HLD) was first recorded in the classic Chinese medicine book *Treatise on Febrile Diseases* (Chinese name: *Shanghan Lun*). It is composed of *Coptis chinensis* Franch. (Chinese name: *Huanglian)*, *Panax ginseng C. A.* Mey. (Chinese name: *Renshen*), *Zingiber acuminatum* Valeton (Chinese name: *Ganjiang*), *Cinnamomum cassia* (L.) J.Presl (Chinese name: *Guizhi*), *Pinellia ternata* (Thunb.) Makino (Chinese name: *Banxia*), *Ziziphus jujuba* Mill. (Chinese name: *Dazao*), and *Glycyrrhiza uralensis* Fisch (Chinese name: *Gancao*) (Li et al. 2021). Modern pharmacological studies have shown that many active ingredients in HLD, such as polysaccharides, flavonoids, alkaloids, and trace elements, have regulatory autophagy, anti-angiogenic, anti-inflammatory, and anti-tumor effects (Luo et al. 2021, Xu et al. 2021b, Zhang et al. 2021a). Moreover, the efficacy of chronic glomerulonephritis treated with HLD in addition to conventional Western medicine is more accurate and the improvement of renal function is more obvious, which may be related to the mechanism of regulating the immune function of the body and reducing the inflammatory response (Chen et al. 2021). However, the mechanism of action of HLD for HSPN has not been studied.

The etiology and pathogenesis of HSPN are not well defined yet, but antigens such as viruses, bacteria, or fungi that stimulate the mucosal immune system to increase the deposition of polymorphic IgA and impaired clearance are the main risk factors for the occurrence of HSPN (Shi et al. 2019). In addition, kidney damage caused by the inflammatory reaction of renal tissue is an important reason for the progression of the disease to end-stage disease (Heineke et al. 2017). It has been demonstrated that the NF-κB/NLRP3 signaling pathway plays a key role in the renal immune inflammatory response and in the development of several diseases, and that activation of this signaling pathway results in the production of multiple inflammatory factors that further amplify inflammation (Huang et al. 2021, Xu et al. 2021a). Effective modulation of the NF-κB/NLRP3 signaling pathway would be an important breakthrough in the treatment of HSPN. Therefore, this study will investigate the effect mechanism of HLD on HSPN from the perspective of the NF-κB/NLRP3 signaling pathway, and further provide a new basis for its clinical application and development.

Given the above, this experiment used the method of bovine serum albumin (BSA) + lipopolysaccharide (LPS) + carbon tetrachloride (CCl_4) to establish the HSPN mouse model. The interventional effects of HLD on biochemical indices, renal injury, and expression of factors related to the NF-κB/NLRP3 signaling pathway in HSPN mice were experimentally observed to reveal the efficacy effects and targets of HLD on HSPN. Furthermore, the effects of HLD-containing serum on cell activity and the expression of the NF-κB/NLRP3 signaling pathway-related factors of IgA-induced cells were also analyzed. The potential regulatory role of HLD in the treatment of HSPN was analyzed to provide a possibility for HSPN treatment.

MATERIALS AND METHODS

Animal's experiments

SPF-grade Kunming female mice from Chengdu University of Traditional Chinese Medicine (No. SCXK (Chuan) 2019-11, Chengdu, China) were 2 months old and had body masses of 23-27 g. Mice were fed and watered ad libitum, housed at a temperature of 22±2° C, humidity of 50±20%, with

a 12/12 h light/dark cycle, and the laboratory was cleaned and disinfected regularly. All studies followed the recommendations of the Guide for the Care and Use of Laboratory Animals and conformed to the regulations of the Sichuan Provincial Laboratory Animal Management Committee (No. 20230526002), and every effort was made to minimize animal suffering and reduce the number of animals used in experiments.

Preparation of HLD

Huanglian (9 g), *Renshen* (10 g), *Ganjiang* (3 g), *Guizhi* (3 g), *Banxia* (9 g), *Dazao* (6 g), *Gancao* (6 g). Take the above herb, add 10 times the amount of distilled water, decoct twice for 2 hours each time, filter, combine the filtrate and prepare a raw drug concentration of 0.84 g/mL concentrate, cold, sealed, and store at 4°C away from light. Prepared by Chengdu University of Traditional Chinese Medicine.

Experimental design

After 1 week of adaptive feeding, mice were randomly divided into 6 groups: control group (Con, *n*=6), model group (HSPN, *n*=6), low-dose group of Huanglian Decoction (HSPN+HLD-L, 7.5 g/kg, *n*=6), medium-dose group of Huanglian Decoction (HSPN+HLD-M, 15 g/kg, *n*=6), high-dose group of Huanglian Decoction (HSPN+HLD-H, 30 g/kg, *n*=6), positive drug group (mycophenolate mofetil, HSPN+MMF, 0.3 g/kg, *n*=6). Except for the Con group, all other groups referred to the modeling method reported in the literature (Jiang et al. 2019): the mouse HSPN model was established by bovine serum albumin (BSA, Sigma) + lipopolysaccharide (LPS, Sigma) + carbon tetrachloride (CCl_4, Nanjing Dingde Biotechnology Co., LTD). 10% immunogenic BSA prepared in distilled water and gavaged in mice at 4 mL/kg every other day for 8 weeks; subcutaneous injection of castor oil 0.3 mL + CCl_4 0.1 mL once a week for 9 weeks. 0.025% LPS was prepared with 0.9% NaCl solution and injected 0.2 mL into the tail vein at weeks 6, 8, 10, and 12. Meanwhile, 25% dried ginger water was instilled 10 mL/kg every other day starting at week 9 until the end of week 12. The Con group was given equal amounts of distilled water by gavage, equal amounts of 0.9% sodium chloride solution by subcutaneous injection and tail vein injection, and the ambient temperature was normal room temperature. After 12 weeks, mice showed hematuria and proteinuria, indicating successful modeling. Then, the Con and HSPN groups were gavaged with distilled water at 0.1 mL each; the HSPN+HLD-L, HSPN+HLD-M and HSPN+HLD-H groups were gavaged with HLD (7.5 g/kg, 15 g/kg and 30 g/kg, respectively) and the HSPN+MMF group was gavaged with MMF (0.3 g/kg, MedChemExpress, China), the total gavage was 4 weeks.

HLD-containing serum preparation

Ten SD rats, size 6-8 weeks, weighing approximately (200±20) g, were provided by Chengdu University of Traditional Chinese Medicine (No. SCXK (Chuan) 2019-11, Chengdu, China). The rats were randomly divided into control group (negative serum control group, *n*=5) and HLD group (drug-containing serum group, *n*=5). Rats in the control group were gavaged with saline, rats in the HLD group were gavaged with HLD (15 g/kg) and administered continuously by gavage for 7 d. Blood was collected from the abdominal aorta 1h after the last dose, centrifuged, and the supernatant was inactivated by water bath at 56°C for 30 min, filtered through 0.22 μm microporous membrane, and stored at -20°C.

Extraction of serum IgA1 from HSPN mice

Jacalin binding protein was extracted by Jacalin-Agarose affinity chromatography (Vector

Laboratories, USA), and the Jacalin-binding protein was then separated by Sephacryl S-200 HR molecular sieve column (Amersham Biosciences, UK) to obtain IgA1, and the monomeric IgA1 (mIgA1) was thermally polymerized to polymeric IgA1 (aIgA1) (Dai et al. 2016).

Cell culture

Mouse glomerular thylakoid cells (SV40 MES 13, Procell Life, Wuhan, China) were cultured in RPMI1640 medium (Gibco, USA) containing 10% fetal bovine serum and incubated in an incubator at 37°C with 5% CO_2 to obtain log phase cells routinely for experiments.

CCK-8 assay for cellular activity

The cells were divided into the following groups: HLD-containing serum group (0, 5%, 10%, 15%, 20%), and the maximum non-toxic concentration of HLD on SV40 MES 13 cells were screened using the CCK-8 method (Li et al. 2022) and recorded as HLD-A0. Subsequently, groupings were set: control group (Con, normal culture), IgA1 induction group (IgA1, 0.5 mg/mL), IgA1 induction + negative serum group (IgA1+NC, 0.5 mg/mL + 5%), IgA1 induction + HLD-A0 group (IgA1+ HLD-A0, 0.5 mg/mL + 5%), and IgA1 induction + HLD-A0 + PMA (NF-κB agonist) group (IgA1 + HLD-A0 + PMA, 0.5 mg/mL + 5% + 100 nmol/L), and the cell activity of each group was detected by CCK-8. Cells of logarithmic growth phase (SV40 MES 13 cells) were selected, trypsin digested, made into cell suspension, centrifuged, supernatant discarded, cells were resuspended by adding medium (10% FBS), incubated (37°C, 5% CO_2) for 24 h, then IgA1, HLD-containing serum or PMA was added and incubated for 48 h. 10 μL CCK-8 reagent (Abcam, UK) was added to each well, and incubated in an incubator for 2 h. The absorbance (A) value of each well was detected at 450 nm using an enzyme marker (spectra max PLUS 384, Molecular Devices).

Biochemical analysis

Mice were executed and blood was collected 24 h after the last dose, after centrifugation at 4°C and 3000 r/min for 10 min, the upper serum was collected and the levels of total protein (TP), albumin (ALB), blood creatinine (CRE), and blood urea (URA) in the serum were measured according to the kit instructions (Nanjing Jiancheng, China). In addition, the levels of IL-17, IL-18, TNF-α, and IL-1β in serum and cells were measured by enzyme-linked immunosorbent assay (ZCIBIO Technology Co., Ltd., Shanghai, China), and each sample was repeated three times.

Histopathological examination

The kidney tissues of mice were taken, fixed with 4% paraformaldehyde, embedded in paraffin, cut into 5-μm-thick sections, stained with hematoxylin-eosin (H&E, Beyotime, Beijing) and Periodic Acid-Schiff (PAS, Beyotime, Beijing), respectively, and the morphological changes of kidney histology were observed under the microscope (BA210Digital, Motic China) in each group of mice.

Immunohistochemical staining

Kidney tissues were fixed in 4% paraformaldehyde for 24 h and tissue sections (5μm) were prepared. Sections were placed at room temperature for 1h and soaked twice in xylene for 10 min each time, followed by gradient ethanol for 5 min for each treatment. Microwave antigen repair, 10% sheep serum for closure, after removing the closure solution and adding primary antibodies of IgA, IgG, and C3 (1:100 dilution, Abcam) were incubated overnight at 4°C, and secondary antibody working solution was added dropwise the next day. The next day, the secondary antibody working solution was added dropwise and incubated at 37°C for 30 min, DAB was used for color development, hematoxylin was used

for counterstaining, dehydrated and sealed slices were observed under the microscope, and optical density (OD) was analyzed by Image-Pro Plus version 6.0 image analysis system.

Immunofluorescence staining

Mouse kidneys tissues fixed with 4% paraformaldehyde were paraffin-embedded and made into 4-μm-thick frozen sections, rinsed 3 times in PBS buffer for 3min each time, incubated dropwise with FITC-labeled p65 antibody (1:50, Abcam) for 30 min at 37°C, rinsed 3 times in PBS buffer, sealed, and the expression of p65 was observed under a fluorescence microscope (BZ-X800E, KEYENCE).

Western blot analysis

Total cellular and kidney tissues proteins were extracted using RIPA lysate (BC3711, Solarbio, Beijing, China) and protein concentrations were detected using BCA (ab102536, Abcam, Cambridge, UK). Equal amounts of protein samples were separated by 12% SDS-PAGE and transferred to polyvinylidene fluoride membranes and then closed with 5% skim milk (Sigma) for 1 h. Subsequently, the samples were incubated with primary antibodies against IκBα, p-IκBα, NLRP3, caspase-1, IL-1β (all dilution ratio 1:1000, Abclonal), β-actin (dilution ratio 1:2000, Abclonal), or LaminB (dilution ratio 1:2000, Abclonal) were incubated overnight at 4°C, followed by incubation with horseradish peroxidase (HRP)-coupled secondary antibodies (1:3000) at room temperature for 1 h. The proteins were developed in the ECL darkroom and the developed proteins were imaged using a Bio-Rad full-function imaging system (Thermo Fisher Scientific) to acquire images, and Image-ProPlus to analyze the optical density with β-actin or LaminB as internal reference, and the results were expressed as the relative expression of the target protein.

Statistical analysis

Data were statistically analyzed using SPSS 22.0 statistical software (IBM Corp, Armonk, New York, USA), and datas were expressed as mean ± standard error of the mean (SEM), and one-way ANOVA of variance was used to analyze the differences among multiple groups, post-hoc Tukey tests were utilized for all post-hoc analyses, with $P<0.05$ indicating that the differences were statistically significant.

RESULTS

Effect of HLD on biochemical indexes of HSPN mice

First, the levels of TP, ALB, CRE, URA, IL-17, IL-18, TNF-α, and IL-1β were analyzed to assess the effects of HLD on biochemical parameters in HSPN mice. Compared with the Con group, the levels of TP and ALB in serum were significantly decreased in the HSPN group ($P<0.01$), and the levels of CRE, URA, IL-17, IL-18, TNF-α, and IL-1β in serum were significantly increased in the HSPN group ($P<0.01$). Compared with the HSPN group, the levels of TP and ALB in serum were observably increased ($P<0.01$), while the levels of CRE, URA, IL-17, IL-18, TNF-α, and IL-1β were significantly decreased in the HSPN+HLD-M, HSPN+HLD-H, and HSPN+MMF groups ($P<0.01$). These results suggest that HLD significantly improved the clinical characteristics of HSPN mice (Figure 1).

Effect of HLD on kidney injury in HSPN mice

To observe the effect of HLD on kidney injury in HSPN mice, we performed histopathological examination of the mice. The results showed that compared with the Con group, the renal tissue vascular bulb saw proliferation of thylakoid cells and increased cellular components in the tubulobulb in HSPN mice (Figure 2a), and the area of positive expression

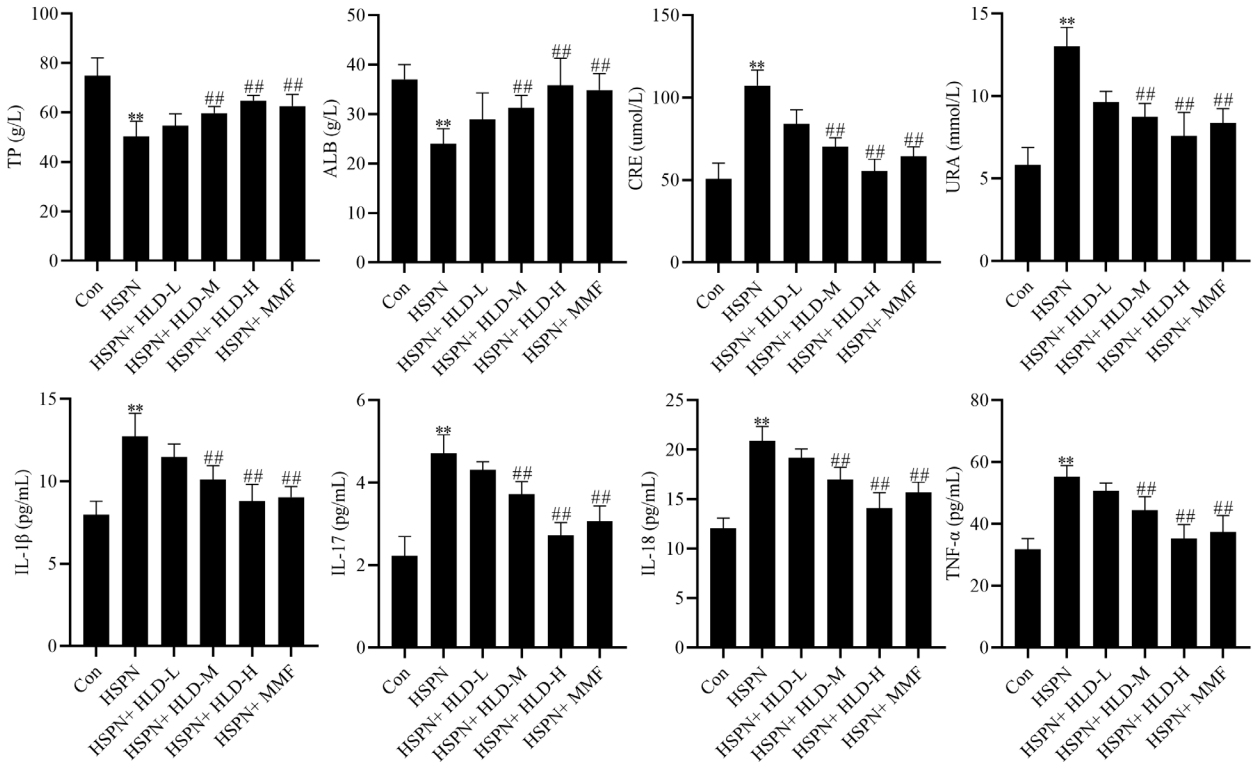

Figure 1. Effect of HLD on biochemical indexes of HSPN mice. The levels of total protein (TP), albumin (ALB), blood creatinine (CRE), blood urea (URA), interleukin-1β (IL-1β), interleukin-17 (IL-17), interleukin-18 (IL-18), and tumor necrosis factor-α (TNF-α) in serum of mice. The data are expressed as the mean ± SEM. Compared with the Con group, $^{**}P<0.01$; compared with the HSPN group, $^{\#\#}P<0.01$. Con: control group; HSPN: model group; HSPN+HLD-L: low-dose group of Huanglian Decoction; HSPN+HLD-M: medium-dose group of Huanglian Decoction; HSPN+HLD-H: high-dose group of Huanglian Decoction; HSPN+MMF: mycophenolate mofetil group.

of PAS staining in renal tissue was markedly increased ($P<0.01$, Figure 2b, f). In addition, the expression of IgA, IgG, and C3 was significantly increased in the kidney tissues of HSPN mice, and the positive cells were stained brownish yellow ($P<0.01$, figure 2c to e, g to i). However, there was a small increase in the intravascular bulb cellular component of the renal tissue, the perithelium of the renal tissue was more intact, the area of positive expression of PAS staining was significantly reduced, and the expression of IgA, IgG, and C3 was dramatically reduced in the HSPN+HLD-H group ($P<0.05$), indicating that the kidney injury in HSPN mice was significantly alleviated after HLD-H treatment.

Effect of HLD on NF-κB/NLRP3 signaling pathway in the kidney of HSPN mice

Next, we analyzed the effect of HLD on the NF-κB/NLRP3 signaling pathway in the kidneys of HSPN mice. p65-specific antibodies identified the expression of p65 in the kidneys of mice, and the data showed that the protein fluorescence intensity of p65 was significantly enhanced in the kidney tissue of mice in the HSPN group compared with the Con group ($P<0.01$), and compared with the HSPN group, the protein fluorescence intensity of p65 was significantly reduced in the HSPN+HLD-L, HSPN+HLD-M, HSPN+HLD-H, and HSPN+MMF groups ($P<0.05$, Figure 3a). Furthermore, the protein expressions of IκBα, p-IκBα, NLRP3, caspase-1, and IL-1β in kidney tissues were analyzed by Western blot. The results showed that the protein expression of p-IκBα, NLRP3, caspase-1, and IL-1β in the

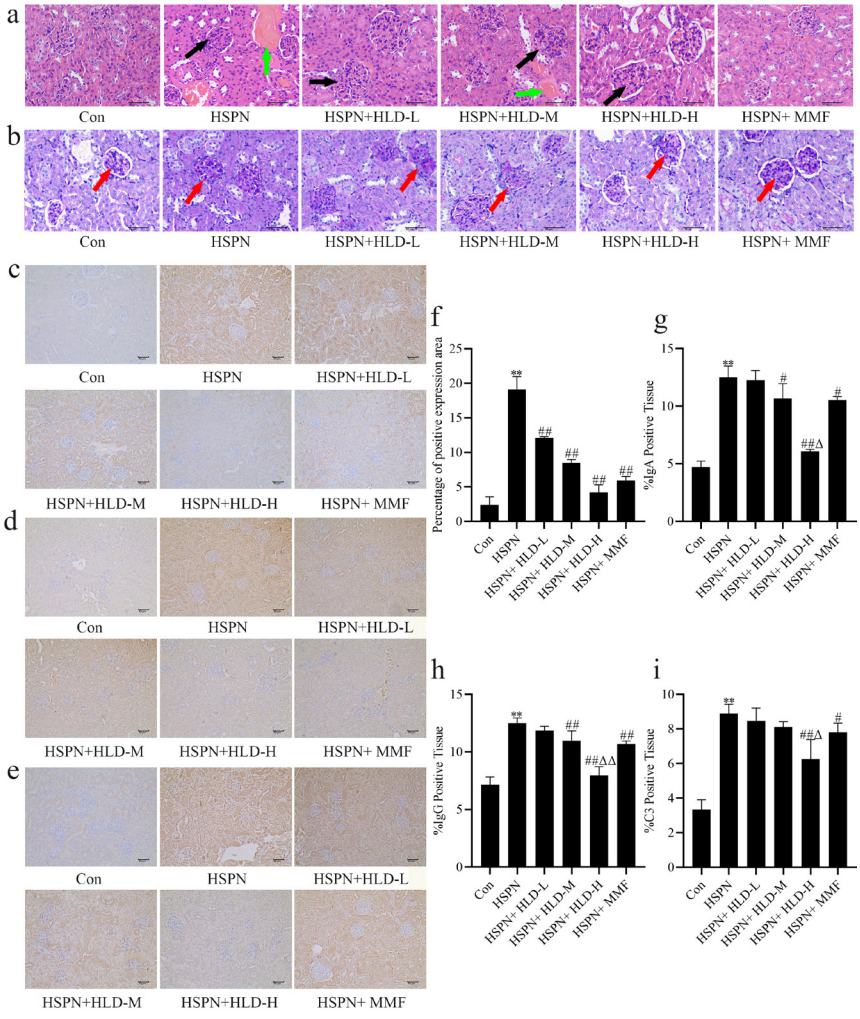

Figure 2 Effect of HLD on kidney injury in HSPN mice. (a) Hematoxylin-eosin (HE) staining observation of kidney tissue (400×, Scale bar, 50 μm), black arrow: mesangial cell proliferation, green arrow: vascular congestion. (b) Periodic Acid-Schiff (PAS) staining observation of kidney tissue (400×, Scale bar, 50 μm), red arrow: Positive expression of PAS staining. (c) Immunohistochemical staining was performed to observe renal tissue IgA (20×, scale bar, 50 μm). (d) Immunohistochemical staining was performed to observe renal tissue IgG (20×, scale bar, 50 μm). (e) Immunohistochemical staining was performed to observe renal tissue C3 (20×, scale bar, 50 μm). (f) Percentage of positive expression area. (G) %IgA positive tissue. (h) %IgG positive tissue. (i) %C3 positive tissue. The data are expressed as the mean ± SEM. Compared with the Con group, $^{**}P<0.01$; compared with the HSPN group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; compared with the HSPN+MMF group, $^{\Delta}P<0.05$ $^{\Delta\Delta}P<0.01$.

kidney tissues of mice in the HSPN group was significantly increased ($P<0.01$), in contrast, the protein expression of p-IκBα, NLRP3, caspase-1, and IL-1β was significantly inhibited in the HSPN+HLD-L, HSPN+HLD-M, HSPN+HLD-H, and HSPN+MMF groups, and the effect of HSPN+HLD-H was more pronounced ($P<0.05$, figure 3b), demonstrating that HLD inhibited the activation of NF-κB/NLRP3 signaling pathway in HSPN mice.

Effect of HLD-containing serum on IgA1-induced SV40 MES 13 cells

We further confirmed the effect of HLD on HSPN. The maximum non-toxic concentration of HLD on SV40 MES 13 cells was first observed, and it was found that there was no statistically significant difference in cell proliferation in the 5% HLD-containing serum group compared to the Con group ($P>0.05$), thus, the concentration of 5% HLD-containing serum was recorded as A0 (HLD-A0) for subsequent experiments. An *in vitro* model of HSPN was constructed by IgA1- induced SV40 MES 13 cells, and HLD-A0 or PMA was used to interfere with the cells. The results showed that cell proliferation, levels of IL-17, IL-18, TNF-α, and IL-1β, and protein expression of IκBα, p-IκBα, NLRP3, caspase-1, IL-1β, and p65 were significantly increased in the IgA1 group compared with the Con group ($P<0.05$). Compared with the IgA1 group, the cell proliferation, levels of IL-17, IL-18, TNF-α, and

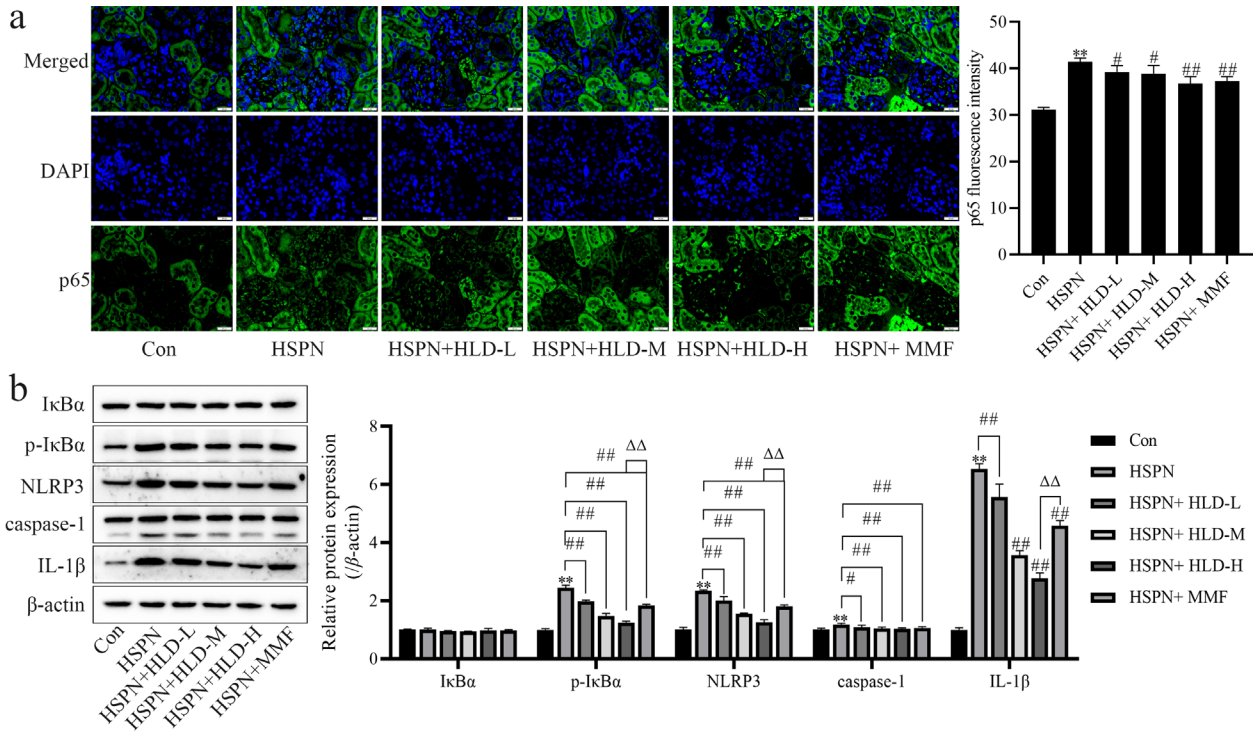

Figure 3 Effect of HLD on NF-κB/NLRP3 signaling pathway in the kidney of HSPN mice. (a) Immunofluorescence staining for p65 expression in renal tissues (40×, scale bars, 20 μm). (b) Relative protein expression of IκBα, p-IκBα, NLRP3, caspase-1, and IL-1β, those in western blot assays were expressed after being normalized to β-actin. The data are expressed as the mean ± SEM. Compared with the Con group, $^{**}P<0.01$; compared with the HSPN group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; compared with the HSPN+MMF group, $^{\Delta\Delta}P<0.01$. DAPI: 4,6-diamidino-2-phenylindole.

IL-1β, and protein expression of IκBα, p-IκBα, NLRP3, IL-1β, and p65 were significantly reduced in the IgA1+ HLD-A0 and IgA1+ HLD-A0+ PMA groups ($P<0.05$). However, the cell proliferation, levels of IL-17, IL-18, TNF-α, and IL-1β and protein expression of IκBα, p-IκBα, NLRP3, IL-1β, and p65 were significantly increased in the IgA1+HLD-A0+PMA group compared to the IgA1+HLD-A0 group ($P<0.05$), as shown in figure 4a, b, and c, suggesting that HLD may inhibit the progression of HSPN by suppressing the activation of NF-κB/NLRP3 signaling pathway.

DISCUSSION

In the present study, we demonstrated that HLD alleviated renal lesions in HSPN mice and inhibited the activation of NF-κB/NLRP3 signaling pathway in HSPN kidneys. Further results showed that HLD significantly attenuated the levels of IgA1-induced inflammatory cytokines and the expression of NF-κB/NLRP3 signaling pathway-related factors in glomerular thylakoid cells. Thus, it is evident that the improvement of HSPN by HLD may be achieved through the NF-κB/NLRP3 signaling pathway.

Previous studies reported that renal damage occurs in 30%-50% of patients with allergic purpura and HSPN is the main cause of death (Bose et al. 2019). HSPN is characterized by hematuria and proteinuria as the main clinical manifestations and its pathological basis is excessive extracellular matrix deposition and thylakoid cell proliferation (Kurt-Şükür et al. 2021). IgA deposition in renal tissue is an important part of renal tissue injury in HSPN (Vaz et al. 2021). Direct immunofluorescence of skin biopsies from children with HSPN revealed

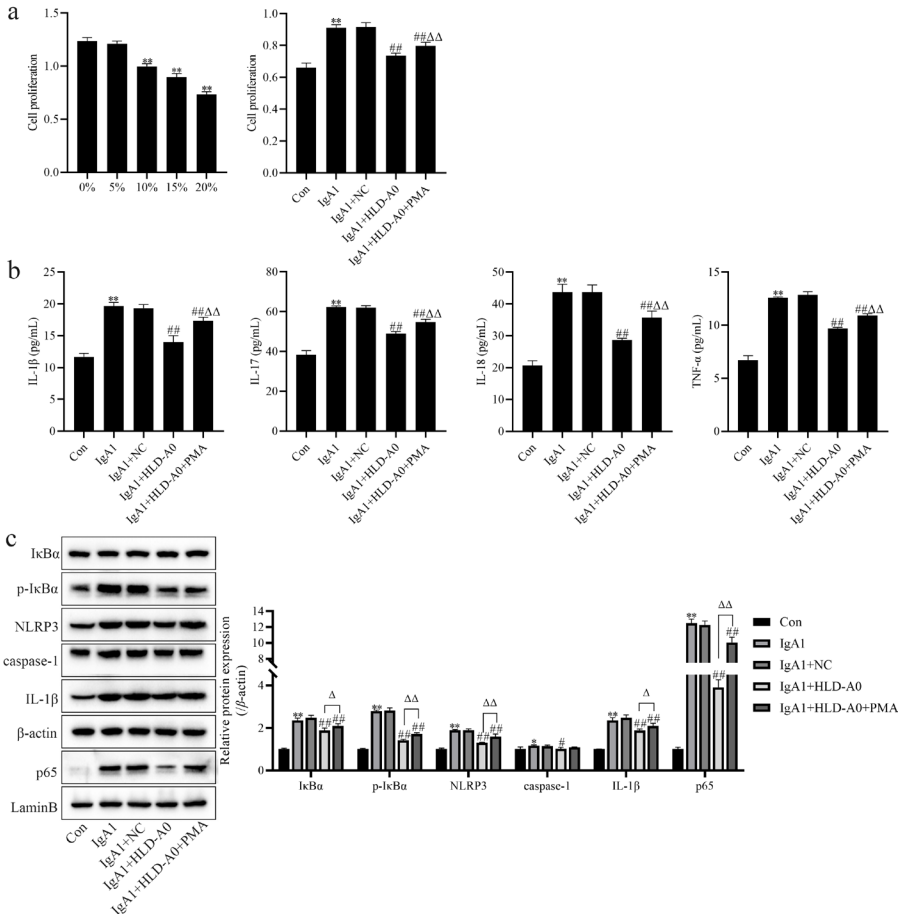

Figure 4 Effect of HLD-containing serum on IgA1-induced SV40 MES 13 cells. (a) CCK-8 assay for cell proliferation. (b) The levels of IL-1β, IL-17, IL-18, and TNF-α in cell. (c) Relative protein expression of IκBα, p-IκBα, NLRP3, caspase-1, IL-1β, and p65, those in western blot assays were expressed after being normalized to β-actin or LaminB. The data are expressed as the mean ± SEM. Compared with the Con group, $^{**}P<0.01$; compared with the IgA1 group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; compared with the IgA1+HLD-A0 group, $^{\Delta}P<0.05$ $^{\Delta\Delta}P<0.01$. Con: control group; IgA1: IgA1 induction group; IgA1+NC: IgA1 induction + negative serum group; IgA1+ HLD-A0: IgA1 induction + HLD-A0 group; IgA1 + HLD-A0 + PMA: IgA1 induction + HLD-A0 + PMA (NF-κB agonist) group.

that IgA deposition was most common (Kim et al. 2021). In addition, C3 is the most abundant complement component in serum, mainly synthesized by macrophages and liver, and plays an important role in both the classical activation pathway and the bypass activation pathway of complement (Ort et al. 2020). The level of C3 gradually decreases with the aggravation of HSPN patients, which eventually leads to immune regulation imbalance (Bose et al. 2019). It is suggested that IgA and C3 play an important role in the pathogenesis of HSPN. In this study, the levels of IgA, IgG, and C3 in the kidneys of the HSPN group were higher than those of the control group, similar to those reported by Su et al (Su et al. 2021), suggesting that IgA, IgG, and C3 were associated with the progression of renal tissue injury in HSPN. We also found that the intravascular bulb cellular component of renal tissue was increased in the HSPN group, and the levels of TP and ALB in serum were significantly lower than those in the control group, demonstrating that renal tissue was damaged in HSPN. However, HLD reversed this situation. This finding suggests that HLD may alleviate renal tissue damage in HSPN.

The NF-κB/NLRP3 signaling pathway has been reported to play an important role in the transcriptional regulation of immune inflammation-related genes (Li et al. 2017). Activation of NF-κB signaling pathway is present in renal parenchymal cells such as podocytes and renal tubular epithelial cells (Markó et al. 2016). When cells are subjected to some stimuli of external origin, gene transcription of NF-κB is induced, and NF-κB then dissociates from

the p50/p65/IκB heterotrimer and translocates to the nucleus, enhancing the transcription of inflammation-related genes and further secreting inflammatory cytokines such as IL-1β and IL-18, but the maturation of IL-1β and IL-18 requires the activation of NLRP3 inflammatory vesicles, which in turn shear, activate and release inflammatory factors (Ma et al. 2022, Sun et al. 2022). Current evidence substantiates that NLRP3 in renal tissue is stimulated to form inflammatory vesicles with caspase-1 and IL-18, which are involved in the inflammatory response process in acute and chronic kidney disease, leading to further kidney tissue damage (Xu et al. 2021a, Yi et al. 2017). In the present study, HLD significantly inhibited the expression of upregulated IκBα, p-IκBα, NLRP3, caspase-1, IL-1β, and p65 in the kidney tissues of HSPN mice and cells, and suppressed the levels of inflammatory cytokines IL-17, IL-18, TNF-α, and IL-1β, indicating that HLD could inhibit the activation of NF-κB/NLRP3 signaling pathway activation, thereby inhibiting the secretion of downstream inflammatory cytokines and attenuating HSPN kidney injury.

Overall, this study found that HLD attenuates renal injury, IgA deposition, and inflammation in HSPN mice, and the mechanism of action may be related to the inhibition of the NF-κB/NLRP3 pathway, which in turn inhibits excessive cellular inflammatory responses. Given the multi-pathway, multi-target, and other action characteristics of TCM, further studies on the mechanism of action of HLD will still follow.

Acknowledgments

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