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ORIGINAL RESEARCH

17(R)-RvD1 Ameliorates Liver Injury in Hyperuricemia Through Inhibiting Pyroptosis via NF-κB Signaling Pathway

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Purpose: The prevalence of hyperuricemia has been increasing worldwide. Most studies have focused on the influences of hyperuricemia on kidney damage and cardiovascular disease. However, the impacts of hyperuricemia on the liver remain unclear. 17(R)-RvD1 (Resolvin D1) plays a crucial role in various pathological conditions associated with inflammation. This study aims to investigate the effects of 17(R)-RvD1 on hyperuricemia-induced hepatic injury.

Methods: Potassium oxonate and hypoxanthine were used to establish a hyperuricemic mouse model and investigate the effects of 17(R)-RvD1 on hyperuricemia and concomitant liver injury. Serum uric acid, xanthine oxidase (XOD), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and inflammatory cytokines levels were assessed. Hematoxylin–eosin (HE), Masson and Sirius Red staining were used to detect histological alterations in the liver. The mRNA and protein expression levels were determined by qRT-PCR and Western blot, respectively. Anti-inflammatory and anti-pyroptosis effects of 17(R)-RvD1 were also observed in LO2 cells exposed to uric acid.

Results: 17(R)-RvD1 administration ameliorated serum uric acid, ALT, AST levels; decreased serum IL-1β, IL-6, TNF-α and IL-18 levels; mitigated hepatic inflammatory responses; reduced hepatic NLRP3, ASC and caspase-1 mRNA expression levels and c-caspase-1, IL-1β and IL-18 levels in hyperuricemic mice. Furthermore, 17(R)-RvD1 administration increased cell viability and reduced LDH release; decreased NLRP3, ASC and caspase-1 mRNA expression levels and Gasdermin D (GSDMD), c-caspase-1, IL-1β and IL-18 levels in LO2 cells exposed to uric acid. Finally, the anti-pyroptosis effects of 17(R)-RvD1 were blocked when the NF-κB signaling pathway was inhibited by BAY 11–7082.

Conclusion: 17(R)-RvD1 possesses anti-hyperuricemic and anti-inflammatory effects, and the underlying mechanism for ameliorating hepatic injury in hyperuricemia is the inhibition of cell pyroptosis via downregulating the NF-κB signaling pathway. 17(R)-RvD1 could serve as an ideal candidate drug and increase options for the treatment of hyperuricemia.

Keywords: 17(R)-RvD1, hyperuricemia, liver injury, pyroptosis, NF-κB

Introduction

Uric acid (UA) is primarily synthesized in the liver modulated by a rate-limiting enzyme named xanthine oxidase (XOD), also referred to as xanthine oxidoreductase. Uric acid is both an antioxidant and a prooxidant, it has been postulated that uric acid could act as a compensatory mechanism, or antioxidant, to protect the body from the flux of pro-oxidants, such as scavenging peroxynitrite.[1] However, uric acid is also well recognized as a pro-inflammatory molecule, existing both as

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a crystal and in a soluble form.[2] Hyperuricemia (HUA) is a metabolic disorder characterized by elevated serum uric acid levels. It occurs due to increased uric acid synthesis, decreased uric acid excretion or a combination of both mechanisms.[3] Owing to lack of uricase for an evolutionary advantage, uric acid cannot be broken down into easily excreted allantoin, making hyperuricemia a relatively unique disease in human.[4] Hyperuricemia is mainly related to lifestyle, drug therapy and genetic factors.[5] Hyperuricemia is widespread, and its prevalence has been rising worldwide. The prevalence of hyperuricemia in the general population in the United States is 21%,[6] and the prevalence in China is 13–25%.[7] Prevalence of obesity is high in diabetes mellitus and is associated with hyperuricemia, and elevated serum uric acid has been shown to be associated with obesity. What's more, there is a significant positive association between serum uric acid and generalized obesity among newly onset diabetes mellitus.[8] Hyperuricemia is speculated to be the second most prevalent metabolic disease following type 2 diabetes in the future. Accumulating evidence has demonstrated that alterations in serum uric acid balance can contribute to the onset of various diseases. Hyperuricemia is correlated with metabolic syndrome,[9] hypertension,[10] non-alcoholic fatty liver disease,[11] chronic kidney injury,[12] heart failure,[13] sleep apnea syndrome,[14] type 2 diabetes.[15] Currently, predominant research on hyperuricemia is concentrated on kidney damage, with comparatively fewer in-depth studies addressing its effects on liver injury. Relevant experimental reports have demonstrated that uric acid can cause hepatic inflammatory cell infiltration,[16] promote lipid accumulation,[17,18] regulate hepatic steatosis,[19] induce liver fibrosis[20] through several pathways. On the one hand, uric acid induces liver injury by upregulating HIF-1α pathway and increasing ROS generation in mouse liver and human LO2 hepatocytes.[21] On the other hand, uric acid induces liver fibrosis through activation of inflammatory mediators and proliferating hepatic stellate cell in mice.[20] However, the molecular mechanisms underlying uric acid-induced liver injury in hyperuricemia have not yet been elucidated.

Pyroptosis, a highly inflammatory mode of programmed cell death, is typically initiated by various pathological triggers and is most commonly observed during intracellular pathogen infections.[22] The common features of pyroptosis comprise cell swelling, swift plasma membrane rupture and pro-inflammatory mediators and cellular contents release, which aggravate inflammation.[23] Pyroptosis occurs through two pathways, one is the canonical pathway, driven by caspase-1 activation,[24] the other is the noncanonical pathway, mediated by caspase-4/5/11 activation.[25] Canonical pyroptosis is initiated when inflammasomes detect a range of external and internal danger signals, for instance, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) followed by the activation of caspase-1, and subsequently activated Gasdermin D (GSDMD).[26] Noncanonical pyroptosis relies on caspase-4/5/11 which can be directly stimulated by lipopolysaccharide (LPS) and PAMPs, then activate GSDMD, the key mediator of pyroptosis, binds to membrane phospholipids and triggers pore formation, leading to cell death.[27] Accumulating studies have indicated that pyroptosis activation is involved in liver injury.[28–30]

Resolvin D1 (RvD1) as a member of the resolvin family of lipid mediators is a potent anti-inflammatory and pro-resolution molecule without resulting in immunosuppression.[31] Its epimer, 17(R)-Resolvin D1 (17(R)-RvD1), exerts comparable functionality to RvD1.[32] 17(R)-RvD1 plays a vital role in multiple pathological processes associated with inflammation. It has been confirmed that 17(R)-RvD1 mitigates pressure overload-induced cardiac hypertrophy and fibrosis via inhibition of the NLRP3 inflammasome.[33] Additionally, experimental findings suggest 17(R)-RvD1 also exhibits strong anti-inflammatory and immunoregulatory properties in conditions such as endometriosis,[34] ultraviolet radiation-caused skin inflammation and oxidative stress,[35] mechanical hypersensitivity in peripheral inflammation,[36] and pulmonary fibrosis.[37] Together, the above information indicates that 17(R)-RvD1 has the capacity to alleviate inflammatory responses during the progression of various diseases. Herein, we examined whether 17(R)-RvD1 could display anti-inflammatory effects in hyperuricemia-induced liver injury and revealed some potent molecular mechanisms, providing valuable references for hyperuricemia treatment.

Materials and Methods

Drugs and Kits

17(R)-RvD1, potassium oxonate, hypoxanthine, uric acid and LPS were purchased from MCE, without additional purification. Uric acid, c-caspase-1 and c-caspase-3 assay kits were purchased from Elab Science. XOD, ALT and

AST detection kits were purchased from Jianglai Biotechnology. The IL-1β, IL-6, TNF-α and IL-18 test kits were purchased from MultiSciences (Lianke) Biotechnology. Hematoxylin–eosin (HE), Masson and Sirius Red staining kits were purchased from Solarbio Life Sciences. The LDH release, the bicinchoninic acid (BCA) assay and CCK-8 kits were purchased from Beyotime Biotechnology.

Animal Model and Experiments

Specific pathogen-free (SPF) grade healthy male ICR mice weighing 18–22 g were used. They were given 7 days to acclimatize to the living environment prior to the formal experiments. The animals were maintained in an air-conditioned room with suitable temperature and humidity and a regular 12 h light/dark cycle, with free access to standard rodent chow and water throughout the experimental period. The animal experiments were all conducted in accordance with the 8th edition National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (2011) and the ARRIVE Guidelines 2.0. All procedures were performed in accordance with the guidelines of the Experimental Animal Ethics Committee of Guangzhou Medical University.

The animal model experiment method was based on previous studies.[33,38,39] In brief, all mice were randomly allocated into three groups: control group, mice in this group were injected intraperitoneally PBS as vehicle; hyperuricemia (HUA) model group, mice in this group were injected intraperitoneally potassium oxonate (PO) (300 mg/kg) combined with hypoxanthine (300 mg/kg) suspension once a day; HUA+17(R)-RvD1 group, 17(R)-RvD1 was administered intraperitoneally in a dose of 2 μg/kg every other day and 1 hour after modeling. The experimental period was extended to 21 days. Following the final administration on the last day, blood samples were collected by retro-orbital puncture and liver tissues were preserved for subsequent experiments.

Cell Culture

Normal human hepatic cell lines (LO2) were maintained in RPMI-1640 medium containing 10% FBS and 1% penicillin-streptomycin and cultured in incubators with humidified air at 37 °C and 5% CO_2. To establish a hyperuricemia inflammatory cell model and investigate the effect of uric acid on hepatocytes, LO2 cells were stimulated with 750 μmol/L uric acid as previous studies described.[21,40] To further investigate the effect on specific pathways, LO2 cells were pretreated with the NF-κB inhibitor BAY 11–7082 at 2 μM and then stimulated with uric acid.

Determination of Biochemicals

The serum uric acid, XOD, ALT and AST levels were determined according to the manufacturer's protocol. Additionally, cell viability, LDH release, IL-1β, IL-6, TNF-α, IL-18, c-caspase-1 and c-caspase-3 levels were measured according to the manufacturer's instructions.

Liver Histological Analysis

For histological evaluation, liver tissues were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and sectioned into 4 μm slices. These sections were then stained using standard hematoxylin and eosin (H&E), Masson and Sirius Red staining, and imaged under an optical microscope for basic morphological analysis. According to the NASH Clinical Research Network criteria,[41,42] the NAFLD activity score has been assessed on a scale of 0 to 8, the components of NAFLD activity score are steatosis (scale 0–3), hepatocellular ballooning (scale 0–2) and lobular inflammation (scale 0–3).

RNA Isolation and Quantitative Real-Time PCR

Total RNA was isolated from the tissues and cells using a commercial RNA isolation kit (Qiagen, Hilden, Germany). The total RNA concentration was measured using a NanoDrop 2000 spectrophotometer. RNA samples were reversely transcribed into single-stranded cDNA using a Reverse Transcription Kit (Applied Biosystems). Quantitative Real-time PCR (qRT-PCR) was performed to assess mRNA expression levels using PowerUP™ SYBR™ Green Master Mix (Thermo Fisher Scientific), with β-actin as an internal reference gene for relative quantification. The primer sequences used in this study were as follows: mouse NLRP3, 5'-ATTACCCGCCCGAGAAAGG-3' and 5'-CATGAGTGTGGCTAGATCCAAG-3'; mouse ASC, 5'-GACAGTACCAGGCAGTTCGT-3' and 5'-AGTCCTTGCAGGTCAGGTTC-3'; mouse caspase-1,

5’-ACACGTCTTGCCCTCATTATCT-3’ and 5’-ATCACCTTGGGCTTGTCTTTCA-3’; mouse URAT1, 5’-AGCTCTTGGACCCCAATGC-3’ and 5’-CTTCAGAGCGTGAGAGTCACACA-3’; human NLRP3, 5’-GCTGGCATCTGGATGAGGAA-3’ and 5’-GTGTGTCCTGAGCCATGGAA-3’; human ASC, 5’-ATCCAGGCCCCTCCTCAG-3’ and 5’-AGAGCTTCCGCATCTTGCTT-3’; human caspase-1, 5’-GAGAAAAGCCATGGCCGACA-3’ and 5’-GCCCCTTTCGGAATAACGGA-3’; mouse/human β-actin, 5’-TCAAGATCATTGCTCCTCCTGAG-3’ and 5’-ACATCTGCTGGAAGGTGGACA-3’.

Western Blot Analysis

Total tissue or cell proteins were extracted using radioimmunoprecipitation (RIPA) lysis buffer and centrifuged at 12,000×g at 4°C for 20 min to obtain the supernatant. The protein concentrations were measured using a BCA protein assay kit. Proteins separated by electrophoresis were transferred from sodium dodecyl sulfate-polyacrylamide gels onto nitrocellulose membranes. The membranes were then incubated with 5% skimmed milk for 2 h at room temperature and subsequently incubated with the designated primary antibodies overnight at 4°C for NF-κB p65 (10,745-1-AP; 1:1000; Proteintech), p-NF-κB p65 (82,335-1-RR; 1:2000; Proteintech) and β-actin (TA-09; 1:1000; Zhongshan Jinqiao Biotechnology). The next day, the membranes were washed thrice and incubated with the appropriate secondary antibodies for 1 h at ambient temperature. Protein bands were scanned and quantified using the Odyssey system (LI-COR).

Immunofluorescence Staining

Immunofluorescence (IF) staining was carried out to determine the GSDMD protein as the same procedures as previously described.[43] GSDMD was stained with anti-GSDMD antibody (AF4012; 1:100; Affinity) and the nucleus was stained with DAPI (Beyotime). The samples were observed by IX73 inverted fluorescence microscope (Olympus).

Statistical Analysis

Data processing was conducted using the GraphPad Prism 9 software. All data were expressed as mean±standard deviation (SD). Differences between and among groups were compared using the Student’s *t*-test and one-way analysis of variance (ANOVA) with Tukey’s post hoc test, respectively. Statistical significance of NAFLD activity score was determined by Mann–Whitney test. $P < 0.05$ was considered statistically significant.

Results

17(R)-RvD1 Ameliorates Serum Uric Acid Levels and Improves Hepatic Function in Hyperuricemia Mice

The experimental protocol, including the modeling and drug administration modes, was depicted in Figure 1A. Initially, we examined serum uric acid levels in hyperuricemic mice. As shown in Figure 1B, the model group exhibited significantly higher serum uric acid levels than the control group after 21 days of potassium oxonate (PO) and hypoxanthine administration, implying the successful establishment of a hyperuricemic mouse model. Serum uric acid levels were substantially decreased in the 17(R)-RvD1 group compared to those in the model group, and the results were consistent with expectations. The liver is an essential organ for several physiological processes. Xanthine oxidase (XOD) catalyses the oxidation of hypoxanthine and xanthine to generate uric acid in the liver. Hyperuricemia occurs due to endogenous uric acid overproduction. As shown in Figure 1C, ELISA kits were used to examine serum XOD levels, which showed upregulation of XOD levels in the serum and 17(R)-RvD1 remarkably downregulated serum XOD levels in hyperuricemic mice. Thus, we conjecture that 17(R)-RvD1 is a potential candidate for hyperuricemia prevention and treatment. However, this requires further experimental validation. Uric acid could cause liver injury, mainly manifested by abnormalities in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). We measured ALT and AST levels in the mouse serum (Figure 1D and E). PO and hypoxanthine administration increased serum ALT and AST levels in hyperuricemic mice compared with normal control mice, whereas 17(R)-RvD1 treatment group mice exhibited reduced serum ALT and AST levels compared with the model group. Therefore, our results demonstrated that 17(R)-RvD1 could mitigate hyperuricemia-induced liver injury.

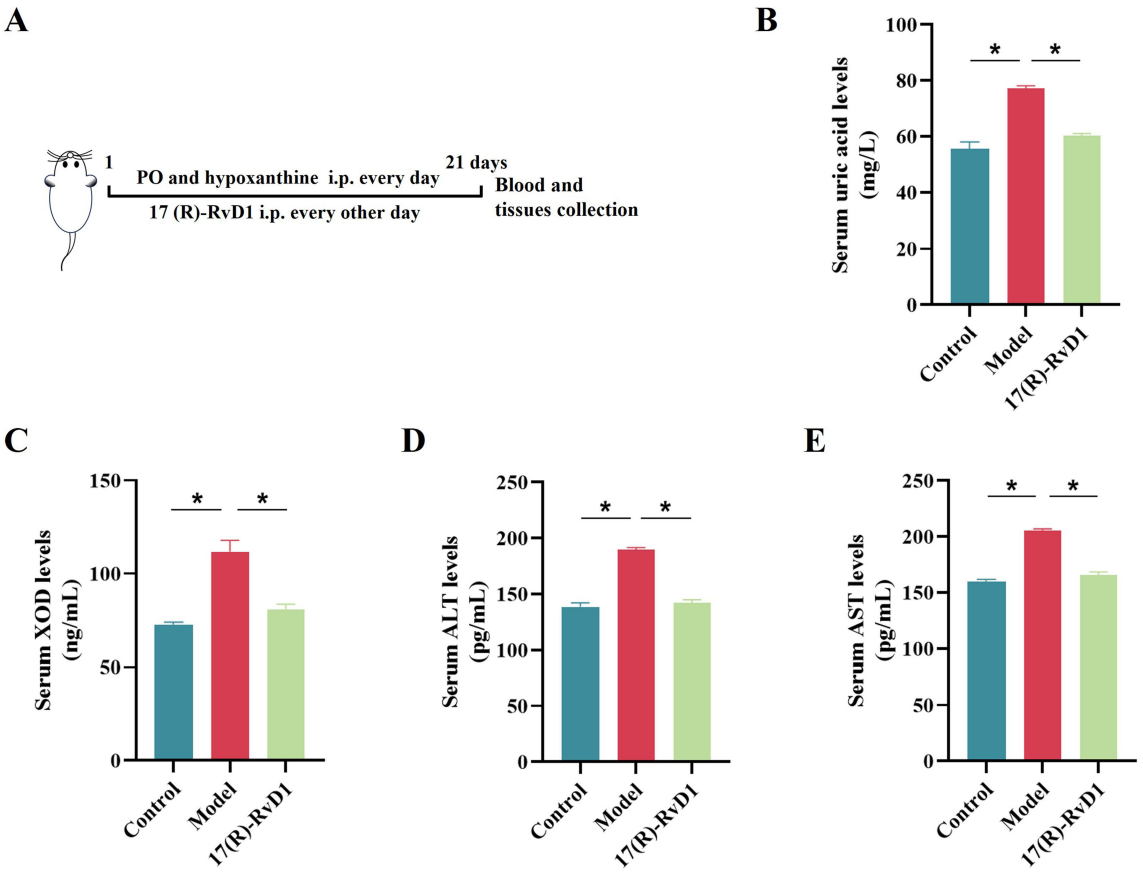

Figure 1 17(R)-RvD1 decreases serum uric acid, XOD, ALT and AST levels in hyperuricemia mice. (**A**) Experimental scheme: PO and hypoxanthine were injected intraperitoneally once a day, 17(R)-RvD1 was administered intraperitoneally every other day after modeling. The mice were euthanized on day 21 for subsequent analysis. (**B**) Uric acid, (**C**) XOD, (**D**) ALT and (**E**) AST levels in mice serum.
Notes: Values are presented as the means±SD (n=5). *P<0.05 was considered to be statistically significant.
Abbreviations: PO, potassium oxonate; XOD, xanthine oxidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

17(R)-RvD1 Decreases Serum Inflammatory Cytokines in Hyperuricemia Mice

Uric acid stimulates inflammatory mediators and induces inflammation. Hence, to explore the underlying mechanisms of 17(R)-RvD1 in alleviating hyperuricemia-induced liver damage, the systemic pro-inflammatory cytokines expression levels in the serum of hyperuricemic mice were detected using ELISA kits for the first step. As shown in Figure 2, compared with the normal control group, serum pro-inflammatory cytokines IL-1β (interleukin-1β), IL-6 (interleukin-6), TNF-α (tumor necrosis factor-α) and IL-18 (interleukin-18) were significantly increased in hyperuricemic mice, whereas administration of 17(R)-RvD1 significantly reversed hyperuricemia-induced elevation in serum IL-1β, IL-6, TNF-α and IL-18 levels in hyperuricemic mice. These results indicated that 17(R)-RvD1 might protect against liver injury caused by hyperuricemia by inhibiting the expression of inflammatory cytokines.

17(R)-RvD1 Mitigates Hepatic Inflammatory Responses in Hyperuricemia Mice

Histopathological analysis of the hepatic tissues of hyperuricemia mice was performed and the photomicrographs of liver tissues for all experimental groups were displayed in Figure 3A and B. The pathological results showed mice in the control group retained a normal liver tissue microstructure, with normal hepatic lobules and hepatocytes morphology, without obvious inflammatory responses. Compared with the control group, the livers of mice in the hyperuricemia model group showed histological alterations, including lipid deposition and inflammation. However, the livers of hyperuricemic mice treated with 17 (R)-RvD1 displayed restoration of normal hepatic histology and improved inflammation compared to the model group. The secretion of inflammatory cytokines regulates the production and migration of inflammatory cells. Accordingly, we performed

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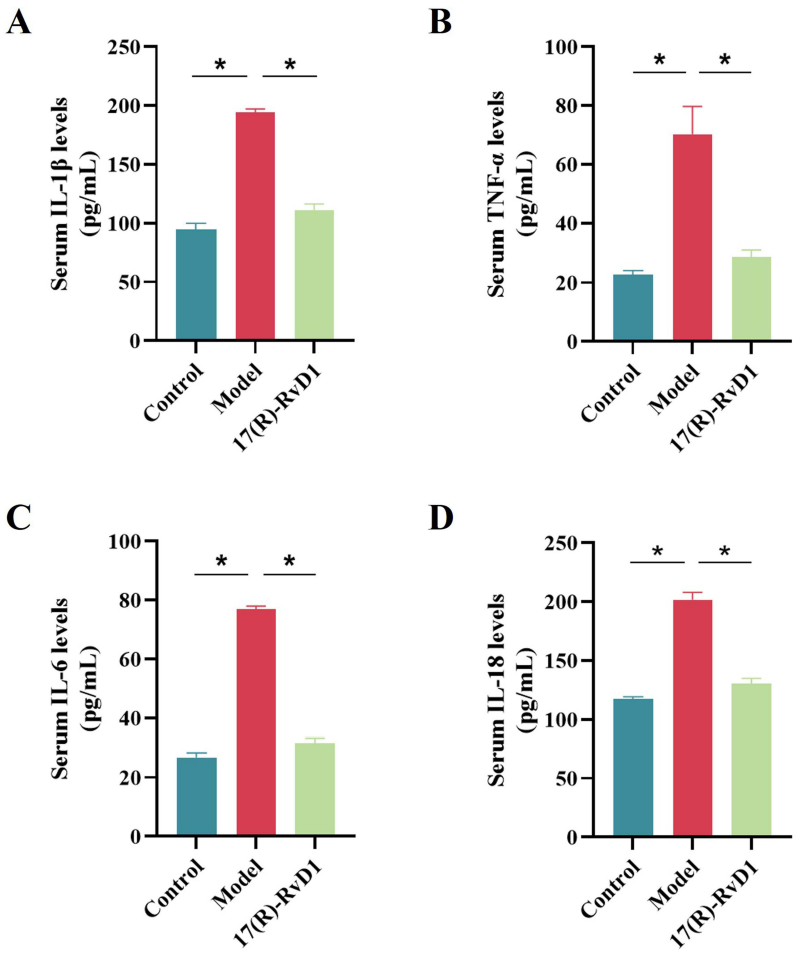

Figure 2 Effects of 17(R)-RvD1 on serum inflammatory cytokines levels in hyperuricemia mice. (**A**) IL-1β levels in mice serum detected by ELISA kit. (**B**) TNF-α levels in mice serum detected by ELISA kit. (**C**) IL-6 levels in mice serum detected by ELISA kit. (**D**) IL-18 levels in mice serum detected by ELISA kit.
Notes: Values are presented as the means±SD (n=5). *P<0.05 was considered to be statistically significant.
Abbreviations: IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; IL-18, interleukin-18.

qRT-PCR analysis of inflammatory molecules, including NLRP3, ASC and caspase-1 in mouse livers to explore the possible effects of 17(R)-RvD1 on pyroptosis. As shown in Figure 3C–E, compared with the normal control group, mRNA levels of NLRP3, ASC and caspase-1 were significantly increased in the livers of hyperuricemic mice, and after 17(R)-RvD1 treatment, the mRNA levels of the above three inflammatory cytokines in mouse livers were all decreased. Next, we tested the levels of downstream products of the NLRP3 inflammatory pathway, c-caspase-1, IL-1β and IL-18, in the livers of mice using corresponding ELISA kits. The results in Figure 3F–H showed 17(R)-RvD1 inhibited hepatic pyroptosis-related inflammatory factor expression in the livers of mice with hyperuricemia. These results suggested that 17(R)-RvD1 ameliorated hyperuricemia-induced liver injury by suppressing pyroptosis. In addition, we found that the expression of URAT1, a urate transporter protein, in the kidney was not affected by 17(R)-RvD1. That's to say, 17(R)-RvD1 did not affect uric acid excretion (Figure S1).

17(R)-RvD1 Attenuates Uric Acid-Induced Hepatocyte Injury in LO2 Cells Exposed to Uric Acid

Uric acid-induced LO2 cells were used as a surrogate in vitro model system to confirm the anti-pyroptosis effects of 17(R)-RvD1. LO2 cells were stimulated with uric acid and 17(R)-RvD1. The CCK-8 and LDH release results suggested that uric acid could increase LO2 cells death and LDH release, while 17(R)-RvD1 could attenuate the effects of uric acid (Figure 4A and B). Then cells were lysed and total RNA was extracted to investigate the effects of 17(R)-RvD1 on NLRP3, ASC and caspase-1 mRNA expression levels in LO2 cells. As shown in Figure 4C–E, uric acid exposure

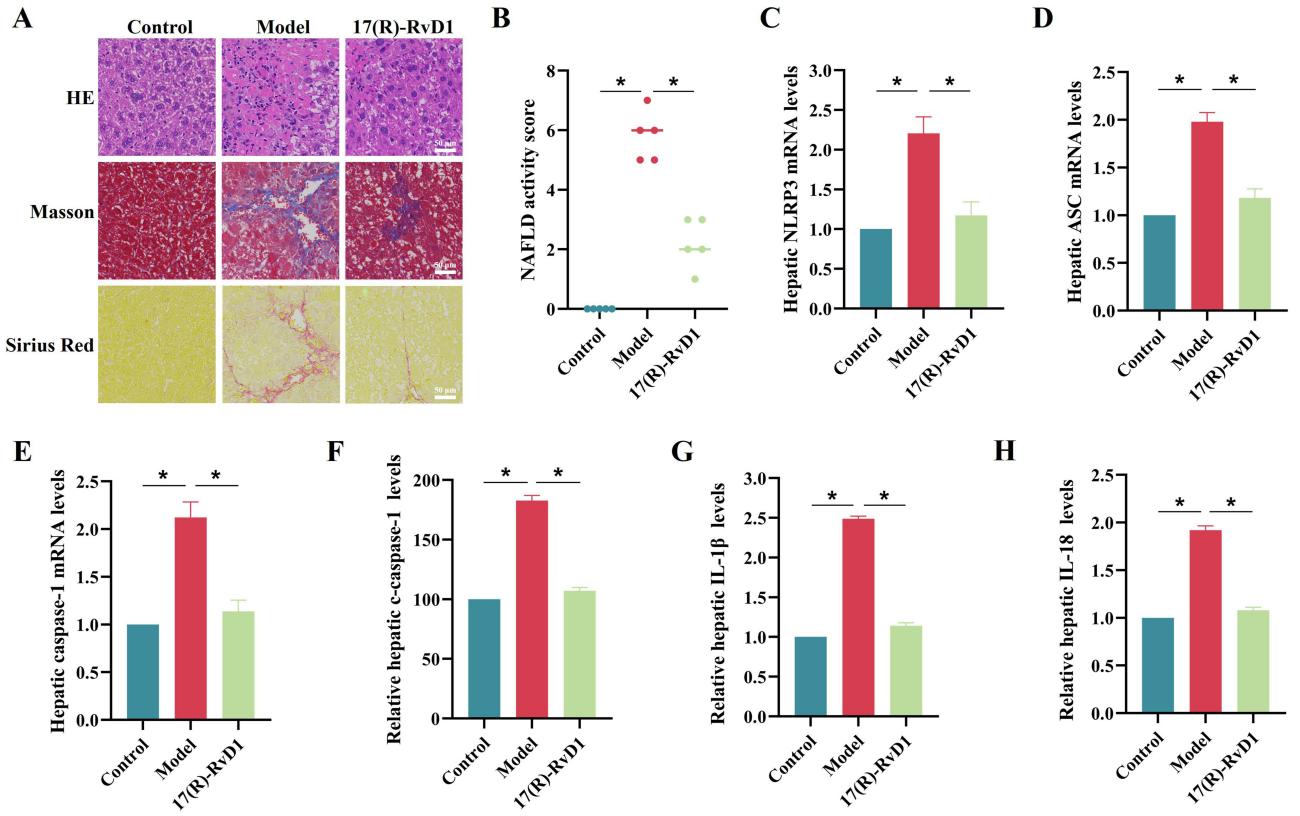

Figure 3 17(R)-RvD1 reduces hepatic inflammatory cytokines levels in hyperuricemia mice. (**A**) Histological analysis of liver tissues in mice. Scale bar 50 μm. (**B**) NAFLD activity score of livers in mice. (**C**) NLRP3 mRNA expression levels of livers in mice. (**D**) ASC mRNA expression levels of livers in mice. (**E**) Caspase-1 mRNA expression levels of livers in mice. (**F**) Hepatic c-caspase-1 levels in mice detected using ELISA kit. (**G**) Hepatic IL-1β levels in mice detected using ELISA kit. (**H**) Hepatic IL-18 levels in mice detected using ELISA kit.

Notes: Values are presented as the means±SD (n=5). *P<0.05 was considered to be statistically significant.

Abbreviations: NLRP3, nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 3; ASC, apoptosis speck-like protein containing a caspase recruitment domain.

resulted in higher mRNA expression of pyroptosis-related cytokines than that in the normal control group, and 17(R)-RvD1 treatment downregulated the mRNA expression levels of these cytokines in LO2 cells.

To explore the effects of 17(R)-RvD1 on pyroptosis-related regulators involved in the modulation of NLRP3 inflammasome activation, we measured c-caspase-1, GSDMD, IL-1β and IL-18 levels in LO2 cells induced by uric acid. Consistent with the results from the in vivo experiments, as shown in Figure 4F–I, c-caspase-1, GSDMD, IL-1β and IL-18 levels were elevated in LO2 cells stimulated with uric acid compared to the normal control group. More importantly, this facilitatory effect was significantly inhibited by 17(R)-RvD1. In addition, we found that 17(R)-RvD1 did not influence cell apoptosis under uric acid stimulation (Figure S2). The above data demonstrated that 17(R)-RvD1 could alleviate hepatocyte injury by reducing pyroptosis-related key regulator expression in uric acid-treated LO2 cells in vitro.

17(R)-RvD1 Inhibits Cell Pyroptosis via Downregulating NF-κB Signaling Pathway in LO2 Cells Exposed to Uric Acid

Uric acid can increase IKK (IκB kinase) and IκB phosphorylation, thereby upregulate NF-κB phosphorylation and activation in hepatocytes.[44] It is well established that the NF-κB signaling pathway is pivotal in initiating the transcription of numerous pro-inflammatory genes, notably, it can also prompt cell pyroptosis. We supposed that 17(R)-RvD1 could regulate cell pyroptosis via NF-κB signaling pathway. Firstly, we treated LO2 cells 17(R)-RvD1 or BAY 11–7082 (an inhibitor of NF-κB signaling pathway) under uric acid treatment. qRT-PCR and Western blot analysis results showed that 17(R)-RvD1 and BAY 11–7082 had similar functions in reducing the levels of c-caspase-1, IL-1β, IL-18 and p-NF-κB levels in LO2 cells exposed to uric acid (Figure 5A–D). What's more, we carried out co-treatment experiments of 17

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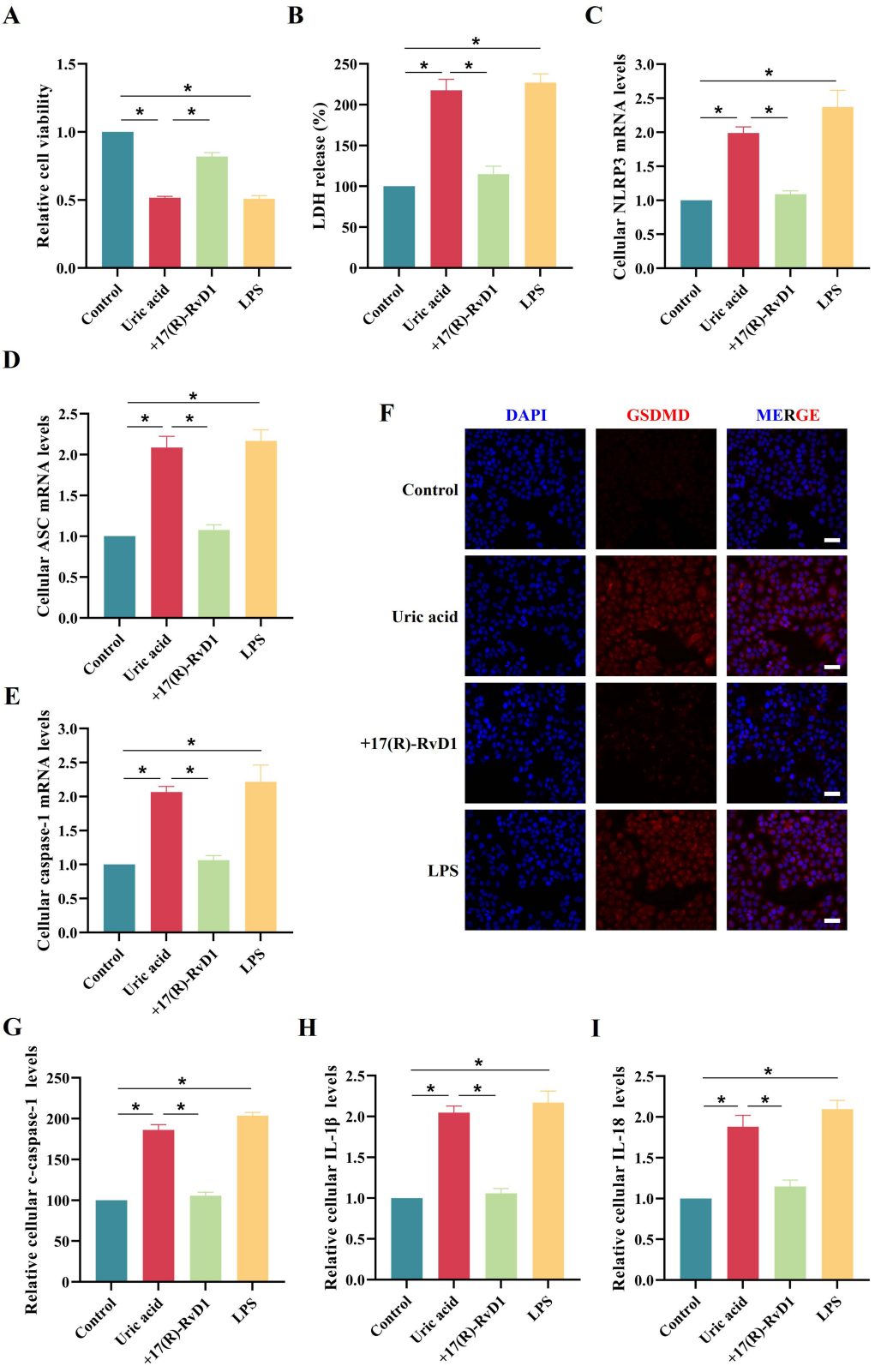

Figure 4 17(R)-RvD1 improves uric acid-induced hepatocyte pyroptosis in LO2 cells. (**A**) Cell viability of LO2 cells. (**B**) LDH release of LO2 cells. (**C**) NLRP3 mRNA expression levels in LO2 cells. (**D**) ASC mRNA expression levels in LO2 cells. (**E**) Caspase-1 mRNA expression levels in LO2 cells. (**F**) GSDMD levels in LO2 cells measured using immunofluorescence. Scale bar 50 μm. (**G**) C-caspase-1 levels in LO2 cells measured using ELISA kit. (**H**) IL-1β levels in LO2 cells measured using ELISA kit. (**I**) IL-18 levels in LO2 cells measured using ELISA kit.

Notes: Values are presented as the means±SD (n=5). *P<0.05 was considered to be statistically significant.

Abbreviation: LPS, lipopolysaccharide.

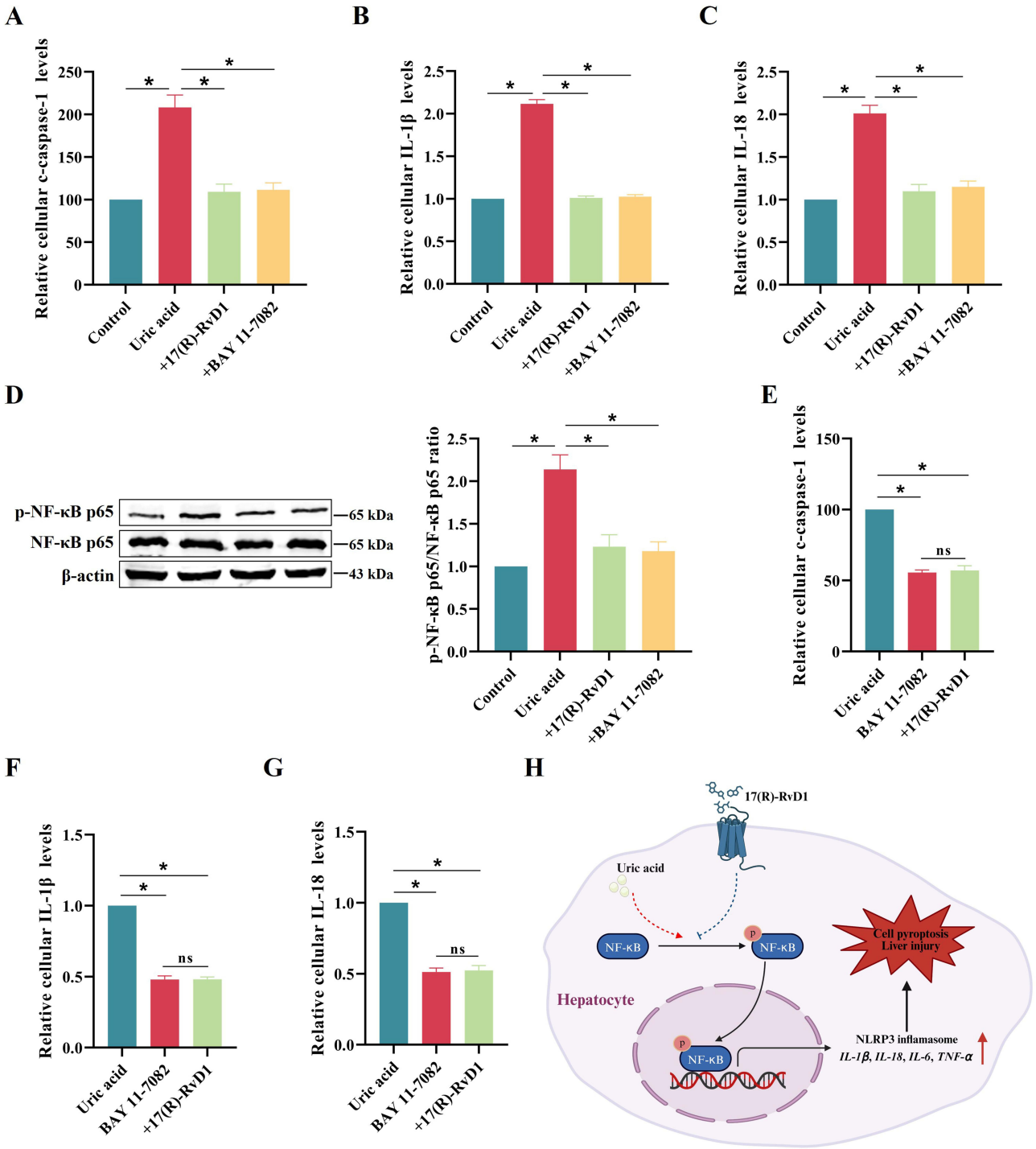

Figure 5 17(R)-RvD1 regulates LO2 cells pyroptosis by inhibiting NF-κB signaling pathway. (**A**) C-caspase-1 levels in LO2 cells exposed to uric acid following treatment with 17(R)-RvD1 or BAY 11–7082. (**B**) IL-1β levels in LO2 cells exposed to uric acid following treatment with 17(R)-RvD1 or BAY 11–7082. (**C**) IL-18 levels in LO2 cells exposed to uric acid following treatment with 17(R)-RvD1 or BAY 11–7082. (**D**) Representative Western blot images were shown on the left, graphic quantification of relative p-NF-κB p65 normalized to NF-κB p65 was shown on the right. (**E**) C-caspase-1 levels in LO2 cells exposed to uric acid following co-treatment with 17(R)-RvD1 and BAY 11–7082. (**F**) IL-1β levels in LO2 cells exposed to uric acid following co-treatment with 17(R)-RvD1 and BAY 11–7082. (**G**) IL-18 levels in LO2 cells exposed to uric acid following co-treatment with 17(R)-RvD1 and BAY 11–7082. (**H**) The speculative mechanism diagram of how 17(R)-RvD1 attenuates liver injury in hyperuricemia created using BioRender.

Notes: Values are presented as the means±SD (n=5). *P<0.05 was considered to be statistically significant.

Abbreviation: ns, no significant difference.

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(R)-RvD1 and BAY 11–7082 in LO2 cells exposed to uric acid. We found that 17(R)-RvD1 could not further inhibit cell pyroptosis after BAY 11–7082 treatment (Figure 5E–G). The results collectively illustrated that 17(R)-RvD1 had a prospective role in ameliorating pyroptosis in uric acid-induced LO2 cells via NF-κB inhibition.

Discussion

More recently, the incidence of hyperuricemia has been increasing, and an increasing number of young people are suffering from hyperuricemia. It seriously endangers the health of people. Allopurinol and febuxostat are the most commonly used urate-lowering drugs. Both allopurinol and febuxostat are generally safe drugs but can have serious adverse effects.[45] Febuxostat has a risk of cardiovascular adverse events and its cardiovascular safety even motivates a FDA-issued public safety alert.[46] Recent study reports that allopurinol may induce severe cutaneous adverse drug reactions and liver injury.[47] Thus, identification of novel products or compounds with high efficacy and safety profiles is crucial. Some studies have demonstrated that uric acid can contribute to hepatic damage. Nevertheless, the molecular mechanisms underlying uric acid-induced liver injury in hyperuricemia remain insufficient exploration and lack comprehensive elucidation. In the present study, we focused on the liver injury in hyperuricemia and first demonstrated that 17(R)-RvD1 decreased serum uric acid levels; downregulated serum XOD, ALT and AST levels; inhibited NLRP3 inflammasome activation and cell pyroptosis to improve hepatic damage in hyperuricemia mice; and resisted cell pyroptosis through inhibiting the NF-κB signaling pathway in LO2 cells exposed to uric acid. This study is meaningful because it evaluated the urate-lowering effects of 17(R)-RvD1 and illustrated the potential molecular mechanisms underlying the alleviation of liver injury caused by hyperuricemia.

Serum uric acid level is the representative indicator in hyperuricemia.[21] In general, serum uric acid levels are modulated by the equilibrium between its production and excretion.[38] Uric acid is primarily synthesized in liver mediated by XOD. Excessive production of uric acid can increase the burden on liver and even induce pathological damage. ALT and AST are usually considered as key biochemical markers used to detect liver dysfunction. Our present study displayed that uric acid, XOD, ALT and AST levels were increased in serum of mice with hyperuricemia, it should be noted that 17 (R)-RvD1 was effective in reversing these serum parameters alterations in mice.

In addition, we found the expression of URAT1 in the kidney was not affected by 17(R)-RvD1. Therefore, the results suggest that 17(R)-RvD1 has therapeutic potential in the treatment of hyperuricemia via the inhibition of uric acid production and alleviation of hyperuricemia concomitant with liver injury in hyperuricemic mice.

Inflammation serves as a key mechanism in both the onset and maintenance of organ damage.[48] We measured the levels of inflammatory cytokines in the serum. These results confirmed that 17(R)-RvD1 markedly reversed the increased production of inflammatory cytokines in hyperuricemic mice, demonstrating that the hepatoprotective effects of 17(R)-RvD1 might be due to its anti-inflammatory property. Inflammatory responses and pathological changes in the liver tissues were observed as notable features of hyperuricemia.[17,18,20,21] Consequently, hepatic histological examinations for further confirmation also illustrated that hyperuricemia-induced lesions in the liver, which could be effectively attenuated by 17(R)-RvD1 administration.

Pyroptosis is a novel type of programmed and pro-inflammatory cell death. NLRP3 inflammasome activation is involved in hepatocyte pyroptosis, both of which are pivotal in liver inflammation. Our results showed that potassium oxonate combined with hypoxanthine induced the upregulation of NLRP3, ASC adaptor, caspase-1, IL-1β and IL-18 in hyperuricemia model mice, and these changes could be significantly altered by 17(R)-RvD1 administration, suggesting that 17(R)-RvD1 might regulate the NLRP3 inflammasome to alleviate liver pyroptosis in hyperuricemic mice. Subsequently, an in vitro cell model was established to explore the effects of 17(R)-RvD1 on hepatocyte pyroptosis. This part of the research was focused on further verifying our assumption that 17(R)-RvD1 could relieve hepatic dysfunction through the inhibition of inflammation and abrogation of pyroptosis in vitro. 17(R)-RvD1 remarkably increased cell viability and decreased LDH release. At the same time, 17(R)-RvD1 decreased the hepatic NLRP3, ASC adaptor and caspase-1 expression levels. Pyroptosis-related proteins levels were measured to investigate the effects of 17(R)-RvD1 on hepatocytes pyroptosis. The c-caspase-1, GSDMD, IL-1β and IL-18 levels were also reduced by 17 (R)-RvD1. What's more, the above functions of 17(R)-RvD1 could be blocked by BAY 11–7082, the inhibitor of NF-κB signaling pathway. Taken together, our findings showed that 17(R)-RvD1 decreased the expression of the NLRP3

inflammasome and pyroptosis-related inflammatory cytokines, and also indicated that 17(R)-RvD1 had a potential role in reducing hepatocyte pyroptosis via inhibiting NF-κB activation in uric acid-induced LO2 cells.

Although the aforementioned study has yielded several promising and innovative findings that favor the prevention and treatment of hyperuricemia, there are still some limitations and enumerate them below. The first is gender differences of the drugs. There are the gender-based differences in how various drugs affect individuals. Only male mice were taken in this study. We would conduct more experiments in the future to explore the effects of 17(R)-RvD1 on female mice. Secondly, we observed that 17(R)-RvD1 could decrease serum uric acid level in mice. However, we could not know whether 17(R)-RvD1 could synergize with other urate-lowering drugs. To address this confusion, we need to carry out more experiments in the future. In addition, Drug absorption, distribution, metabolism and excretion must be assessed to understand the pharmacokinetic and pharmacological effects of 17(R)-RvD1. Combining industrial pharmaceutical technology, such as multiparticulate systems (MPS) with functional coating,[49] to enhance the bioavailability of 17(R)-RvD1 will be a meaningful project. Thirdly, because 17(R)-RvD1 administration and HUA mice modeling were carried out simultaneously, our study could mainly demonstrate the preventive effect of 17(R)-RvD1. Post-induction treatment of 17(R)-RvD1 would mimic clinical scenarios better. Furthermore, trying to collect serum or liver biopsies from hyperuricemia patients to validate our findings in the next research could be needed. Fourthly, we have detected the roles of 17(R)-RvD1 in apoptosis and pyroptosis in hyperuricemia. Several other types of cell death such as ferroptosis would also be worth investigating. At the end, although there is no universally defined minimum group size, group sizes must be large enough to allow for meaningful statistical analysis. The number of animals in each group was 5 in our animal experiments. Although the sample size met the statistical requirements in this study. It would be best to use as many experimental samples as possible in future experiments.

Conclusion

17(R)-RvD1 has the anti-hyperuricemic and anti-inflammatory effects in hyperuricemia, and it can ameliorate liver injury of hyperuricemia and inhibit cell pyroptosis. Mechanistically, 17(R)-RvD1 inhibits cell pyroptosis via downregulating NF-κB signaling pathway (Figure 5H). Our findings provide additional insights into the development of effective drugs for hyperuricemia treatment.

Data Sharing Statement

The datasets supporting the conclusions of this article are included within the article.

Ethics Approval and Consent to Participate

This study was approved by the Animal Care and Use Committee of Guangzhou Medical University.

Author Contributions

All authors have made substantial contributions to this work, encompassing the study design, methodology, data analysis, interpretation and a combination of these areas. They participated in drafting, revising or critically reviewing the manuscript; provided final approval for the version to be published; agreed on the journal for submission; and committed to being accountable for all aspects of the research.

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Disclosure

The authors declare no conflicts of interest in relation to this paper.

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