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Original Article

Anti-inflammatory Effect of Total Saponin Fraction from *Dioscorea nipponica* Makino on Gouty Arthritis and Its Influence on NALP3 Inflammasome*

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ABSTRACT **Objective**: To investigate the mechanism of Chinese herbal medicine *Dioscorea nipponica* for the treatment of monosodium urate crystals-induced gouty arthritis (GA) in rats. **Methods**: Sixty male Wistar rats were divided into 6 groups: normal, model, indomethacin and three total saponin (900, 300 and 100 mg/kg) groups. The liver, kidney and serum levels of lysosomal enzymes, antioxidant capacities, and inflammatory factors were measured. In addition, the mRNA and protein levels of the NALP3 inflammasome components in the mononuclear cells of rats' peripheral blood were analyzed using real-time polymerase chain reaction and Western blotting methods, respectively. **Results**: Total saponins groups could reduce the activities of β-galactosidase, β-N acetyl glucosamine enzyme, β-glucuronidase, acid phosphatase, and malonaldehyde as well as the contents of TNF-α, IL-1β and IL-8 (all $P<0.05$). They could also increase the activities of glutathione peroxidase and total superoxide dismutase (both $P<0.05$). Further studies showed that total saponins groups of high, middle and low doses could all increase the mRNA and protein levels of caspase-1, adapter apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC) and NALP3 in the mononuclear cells of peripheral blood (all $P<0.05$). **Conclusion**: *Dioscorea nipponica* may treat GA by regulating lysosomal enzymes, antioxidant capacities and the NALP3 inflammasome.

KEYWORDS apoptosis-associated speck-like protein containing a caspase-recruitment domain, caspase-1, Chinese medicine, *Dioscorea nipponica*, NALP3

Gouty arthritis (GA), also called urarthritis, is a type of acute rheumatic pathological change caused by the deposition of uric acid in capsular ligaments, synovium, cartilage, sclerotin and other joint tissues. The high concentration of uric acid in the blood is due to a disturbance in purine metabolism.[1] Epidemiological studies have shown that beverages, particularly beer, and diets that contain high amounts of red meat, shellfish and fish are the main causes of GA attacks.[2,3] The worldwide morbidity rate for GA has been increasing in recent years.[4] GA is difficult to be cured. In the development of GA, it is known that the NALP3 inflammasome plays an important role in the morbidity.[5] NALP3 is also known as cryopyrin. NALP3 inflammasomes are mainly expressed in neutrophils, mononuclear macrophages, and some other primary immune cells. These cells are primarily located in the skin, joints, eyes, ears and the inner surface of the gastrointestinal epithelium.[6] The NALP3 inflammasome is a complex composed of NOD-like receptors (NLRs), a pro-inflammatory caspase and the adapter apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC).[7] During the process of a GA attack, monosodium urate crystals (MSU) induce a conformation change in the NALP3 inflammasome that exposes the NACHT domain and triggers oligomerization, thus activating the NALP3 inflammasome. Once the NALP3 inflammasome is activated, caspase-1 promotes the cleavage and maturation of pro-interleukin (IL)-1β.[8] The activated IL-1β is released, and the inflammation is exacerbated because IL-1β can initiate a robust downstream neutrophilic inflammation.[9] As a result,

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inflammatory factors such as tumor necrosis factor α (TNF-α), IL-6, IL-8, etc. are released. The primary pathological feature of gout is the influx of neutrophils into the joint fluid. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytoses the MSU crystals. This process results in membranolysis, generation of oxygen-derived free radicals and the release of lysosomal enzymes, prostaglandin E2, leukotrienes and IL-1.

Currently, the primary Western medicines used for treating GA are the xanthine oxidase inhibitor allopurinol,[10] colchicine which affects many neutrophil functions,[11] and the non-steroidal anti-inflammatory drug indomethacin.[12] However, the use of these agents is limited by the occurrence of complications or severe side effects. *Dioscoreae nipponicae*, a traditional Chinese herb from the rhizome of *Dioscorea nipponica* Makino (RDN), was widely used in clinic for GA treatment. *Dioscoreae nipponicae* was traditionally used to treat rheumatic arthritis, injuries from falling down and other problems.[13] *D. nipponicae* has anti-inflammatory effects, regulates the immune system and provides relief from pain.[14] Our previous studies identified the main components of the total saponins from *D. nipponicae* and some of them were quantified. We also found that *D. nipponicae* is effective in treating GA.[15-18] However, the anti-inflammatory mechanism was not very clear. The present study aimed to determine whether the total saponin fraction from *D. nipponicae* treat GA by influencing lysosomal enzymes, antioxidant capacities, inflammatory factors or the mRNA and protein expressions of NALP3 inflammasome.

METHODS

Reagents

Uric acid, trizma base, glycine, sodium dodecyl sulfate (SDS), sodium deoxycholate were all purchased from Sigma-Aldrich, China. Indometacin (purity>98%) was purchased from Qilin Pharmaceutical Co., Ltd., China. β-Galactosidase kit (Jiemei Gene Medicine Science and Technology Co., Ltd., China). β-N-acetyl glucosamine enzyme (β-NGE) kit, β-glucuronidase kit, glutathione peroxidase kit, total superoxide dismutase kit, malonaldehyde kit, and protein quantitative test kit were all purchased from Nanjing Jiancheng Bioengineering Institute (China). Phosphatase kit was purchased from Beyotime Institute of Biotechnology (China). Rat TNF-α and IL-1β enzyme linked immunosorbent assay (ELISA) kits were purchased from Multi Sciences Biotech Co., Ltd. (China). Rat IL-8 ELISA kit was purchased from Beijing Notlas biotechnology Co., Ltd. (China). Rat peripheral lymphocyte isolation liquid was purchased from Tianjin Haoyang Biological Manufacture Co., Ltd. (China). RNA extraction kit, reverse transcription kit and SYBR Green kit were all purchased from Dalian TAKARA Biotechnology Co., Ltd. (China). The primers were synthesized by Dalian TAKARA Biotechnology Co., Ltd. (China). RIAP extraction kit and bicinchoninic acid protein quantification kit were both purchased from Beijing Comwin Biotech Co., Ltd. (China). Phenylmethylsulfonyl fluoride (PMSF) mother liquid, bull serum albumin, acrylamide, Bis-acrylamide, ammonium persulfate, Tween-20, tetramethylethylenediamine (TEMED), ponceau S, biomphenol blue, CBB R-250, CBB G-250, DL-dithiothreitol (DTT), NP-40 were all purchased from American Amresco Company (USA). Bovine serum albumin (BSA) standard (2 mg/mL), 5× reduction sample buffer, 10× Tris-glycine-SDS electrophoretic buffer, Kaumas coomassie brilliant blue staining solution, 10× Tris-buffered saline Tween-20 (pH 8.0), middle molecular weight protein marker, middle molecular weight pre-stained protein ladder, wet transfer buffer, stripping buffer, Li Chunhong staining solution were all purchased from Beijing Sainuobo Biotechnology Center (China). Nitrocellulose filter membrane and electro-chemi-luminescence were both purchased from American Millipore Company (USA). Protease and phosphatase inhibitors were both purchased from Roche (Basel, Swiss). Methyl alcohol, Huang sulfosalicylic acid, trichloroacetic acid, NaCl were all purchased from Sinopharm Chemical reagent Beijing Co., Ltd. (China). Gout-anti-rabbit IgG (H+L), horseradish peroxidase, gout-anti-mouse IgG (H+L) and rGAPDH antibody were all purchased from Beijing TDY Biotech Co., Ltd. (China). rCaspase1 and rASC2 antibodies were both purchased from Abcam Trading Company Ltd. (England).

Plant Material and Extraction

RDN (Lot No. CT-873021) was purchased from the Heilongjiang Province Drug Company (China). A voucher specimen (hlj-201104) of the herb was authenticated by Prof. FU Ke at the Research Institute of Chinese Medicine, Heilongjiang University of Chinese Medicine. A voucher specimen of the sample has been deposited at the Chinese Medicine Laboratories. The extraction procedure was in accordance with our previous literature.[19]

Determination of RND Content and UPLC/MS Analysis

The content of RDN, calculated according to the content of dioscin, was approximately 55.9%.[19] To standardize the herbal extract chemically, UPLC/MS analysis was performed. Three major compounds in the RDN extract were identified. They were dioscin, protodioscin and pseudo protodioscin.[19]

Animals and GA Modeling

Male Wistar strain rats (200±20 g) were purchased from the Vital River Laboratory Animal Technology Co. Ltd., China, with license No. SCXK (Jing) 2012-0001. The rats were allowed at least 1 week to adaption used for the experiments. The light environment was controlled, with 12 h of light and 12 h of darkness. Feed was purchased from the Experimental Animal Center of Heilongjiang University of Chinese Medicine. The experiment was approved by the Institutional Animal Care Committee of Heilongjiang University of Chinese Medicine (No. 20120516). The method to induce the model referred to previous literature.[17]

Drug Administration

Sixty male Wistar rats were divided into 6 groups: normal, model, the indomethacin groups (3 mg/kg), and groups treated with the total saponin at a high, middle and low doses (900, 300 and 100 mg/kg, respectively). The doses of total saponins from RDN and indomethacin were determined based on the conversions from clinical adult dosages[13] and our preliminary studies.[20] The total saponin and indomethacin doses were given every 24 h for 7 days. The normal and model groups were administered normal saline at the same time. A 10% solution of chloral hydrate was injected into the abdominal cavity to anesthetize the rats 1 h after the intragastric administration of these drugs on the third day. The MSU suspension was injected bilaterally into rats' joints for 5 days to induce GA model.

Blood, Liver and Kidney Tissue Sample Collection

Blood was collected 1 h after the models were induced on the 5th day. The blood was allowed to clot for 1 h at room temperature. The blood serum was obtained after 10 min of centrifugation at a speed of 4000×*g*. The serum was stored at –20 ℃ until assayed. Simultaneously, the liver and kidney samples were rapidly and carefully separated on an ice-cold plate and stored at –80 ℃ for the assays.

Extraction of Mononuclear Cells

The mononuclear cells were extracted according to the procedure provided by the instructions of the manufacture.

Determination of β-Galactosidase, β-NGE, β-Glucuronidase and Acid Phosphatase Levels in Rats' Liver, Kidney and Blood

The determination of the activities of β-galactosidase, β-NGE, β-glucuronidase and acid phosphatase was achieved using kits. The activity of β-galactosidase was tested using an ELISA. The OD values of β-NGE, β-glucuronidase and acid phosphatase activities were all tested using an ultraviolet spectrophotometer at a wavelength in the range of 400–415 nm.

Determination of Glutathione Peroxidase, Total Superoxide Dismutase and Malondialdehyde Levels in Liver, Kidney and Blood

The activities of glutathione peroxidase and total superoxide dismutase and the concentration of malondialdehyde were measured by kits to test the OD values using an ultraviolet spectrophotometer at a wavelength of 532 nm.

Determination of TNF-α, IL-1β and IL-8 Levels in Blood

The TNF-α, IL-1β and IL-8 levels in the blood were quantified using ELISAs. Wavelength of 450 nm was used to test the OD value.

Real-Time-PCR of NALP3, Caspase-1 and ASC

The RNA was extracted using the classical TRIzol reagent method. Reverse transcription was performed using reverse transcriptase and 1 μg RNA to synthesize the cDNA. The PCR amplification was carried out using gene-specific PCR primers synthesized by Dalian Bao Biotechnology Company (China). The sequences of the gene-specific PCR primers and the length of products are summarized in Table 1. The replication procedure followed previous literature.[19]

Western Blot Analysis of NALP3, Caspase-1 and ASC

For every 1×10^7 cells, 1 mL of the lysing solution was added. The cells were fully suspended using a pipette tip and were incubated on ice for 20 min. They were then centrifuged at 4 ℃ at 13,000×*g* for 20 min. After centrifugation, the supernatant was obtained and

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Table 1. Summary of Gene-Specific Real-Time PCR Primer Sequences

Description	Gene bank	Sense primer (5'→3')	Anti-sense primer (5'→3')
NALP3	NM_001191642	TTGTGTGCACAGGGCTAAAG	CCGCAGATCACACTCTTCAA
Caspase1	NM_172322	TGCCTGGTCTTGTGAACTTGGAG	ATGTCCTGGGAAGAGGTAGAAACG
ASC	NM_012762	TTATGGAAGAGTCTGGAGCTGTGG	AATGAGTGCTTGCCTGTGTTGG
rGAPDH	NM_008084	TGCACCACCAACTGCTTAG	GATGCAGGGATGATGTTC

preserved for further testing. The content of the protein was decided according to previous published literature.[19]

Statistical Analysis

All data were expressed as mean ± standard error of the mean ($\bar{x} \pm$SEM). The statistical analysis was performed using a *t*-test for independent samples and one-way analysis of variance (ANOVA) to determine the levels of significance, and Dunnett's *t*-test was used for further analysis. A value of $P<0.05$ was considered statistically significant.

RESULTS

Effects of RDN and Indomethacin on Activities of Lysosomal Enzymes in MSU Crystal-Induced Rats

High levels of the activities of β-galactosidase, β-NGE, β-glucuronidase and acid phosphatase were found in the sera, livers and kidneys of GA rats as compared to normal rats. However, RDN significantly reduced these enzyme activities to values similar to those of the normal animals ($P<0.05$ or 0.01, Table 2).

Anti-oxidant Effects of RDN and Indomethacin in MSU Crystal-Induced GA Rats

Compared with the normal group, the activities of glutathione peroxidase and total superoxide dismutase were both decreased significantly in the sera, livers and kidneys of GA rats, while the level of malondialdehyde was significantly increased. However, RDN significantly regulated these enzymatic activities to values similar to those of the normal animals ($P<0.05$ or 0.01, Table 3).

Effects of RDN and Indomethacin on the Contents of Inflammatory Factors in MSU Crystal-Induced GA Rats

As shown in Figure 1, compared with the normal group, the levels of TNF-α, IL-1β and IL-8 in the sera of the model group animals were increased significantly ($P<0.05$). RDN at the high, middle and low doses significantly decreased the levels of TNF-α, IL-1β and IL-8 ($P<0.01$ or $P<0.05$), as well as indomethacin ($P<0.01$ or $P<0.05$).

Effects of RDN and Indomethacin on mRNA and Protein Expressions of NALP3 Inflammasome in MSU Crystal-Induced GA Rats

As shown in Figure 2, compared with the normal group, the levels of mRNA and protein expression of caspase1 in the mononuclear cells in the model group were both decreased ($P<0.01$). RDN at the high, middle and low doses significantly increased the mRNA ($P<0.01$) and protein expression ($P<0.01$) of caspase-1. Indomethacin also increased the mRNA and protein expression of caspase-1. However, only the effects on protein expression were statistically significant ($P<0.05$).

Compared with the normal group, the levels of mRNA and protein expression of ASC in the serum of

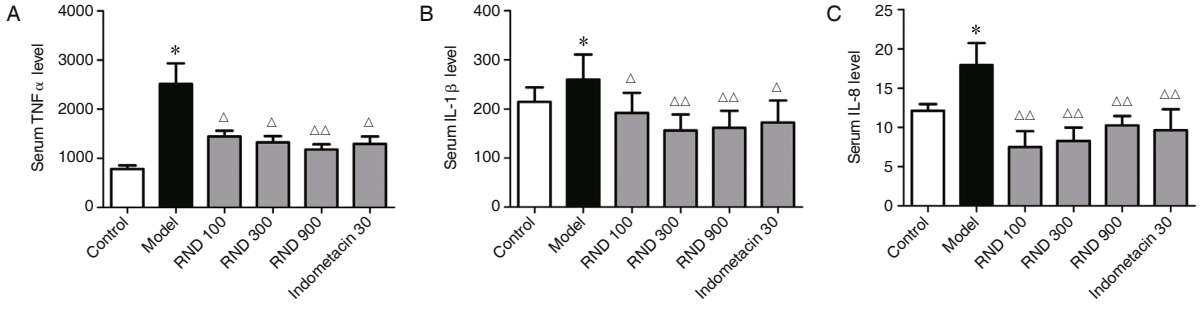

Figure 1. Effects of RDN and Indomethacin on Serum Levels of Inflammatory Factors TNF-a (A), IL-1β (B) and IL-8 (C) In MSU Crystal-Induced GA Rats (n=10, $\bar{x} \pm$SEM)

Notes: *$P<0.05$ *vs*. normal group; △$P<0.05$, △△$P<0.01$ *vs*. model group

Table 2. Effects of RDN and Indomethacin on Activities of Lysosomal Enzymes in MSU Crystal-Induced GA Rats (n=10, $\bar{x} \pm$ SEM)

Parameter	Normal group	Model group	RDN group (100 mg /kg)	RDN group (300 mg /kg)	RDN group (900 mg /kg)	Indometacin group (3mg /kg)
Serum						
β-Galactosidase[a]	0.024 ± 0.037	0.048 ± 0.014*	0.033 ± 0.011	0.027 ± 0.009△△	0.025 ± 0.009△△	0.022 ± 0.004△△
β-NGE (U/L)	31.79 ± 3.532	45.794 ± 4.199*	40.996 ± 9.055	32.498 ± 2.054△	33.967 ± 5.635△	33.178 ± 5.178△
β-Glucuronidase (U/mL)	2.184 ± 0.458	4.302 ± 0.648*	1.151 ± 0.421△△	1.902 ± 0.704△△	0.772 ± 0.234△△	2.334 ± 0.529△△
Acid phosphatase (U/μL)	0.264 ± 0.045	0.351 ± 0.072*	0.295 ± 0.035△	0.265 ± 0.024△△	0.248 ± 0.041△△	0.284 ± 0.057△△
Liver						
β-Galactosidase	0.069 ± 0.024	0.112 ± 0.024*	0.081 ± 0.023	0.072 ± 0.036△	0.067 ± 0.023△△	0.039 ± 0.035△△
B-NGE (U/L)	35.328 ± 4.601	59.897 ± 3.843**	42.213 ± 3.286△	53.228 ± 8.722	41.286 ± 4.311△△	40.028 ± 3.215△△
β-Glucuronidase (U/μg prot)	2.952 ± 0.411	4.097 ± 1.062*	3.772 ± 1.193	3.602 ± 0.904	2.938 ± 0.501△	0.943 ± 0.258△△
Acid phosphatase (U/μg prot)	2.564 ± 0.163	2.985 ± 0.522*	2.582 ± 0.098△△	2.548 ± 0.112△△	2.704 ± 0.143	2.614 ± 0.142
Kidney						
β-Galactosidase	0.124 ± 0.03	0.178 ± 0.038*	0.144 ± 0.043	0.106 ± 0.046△	0.112 ± 0.081△△	0.100 ± 0.013△△
β- NGE (U/L)	1022.554 ± 57.507	1359.869 ± 125.618*	885.959 ± 79.891△△	1027.802 ± 202.693△	915.636 ± 94.408△△	611.159 ± 93.057△△
β-Glucuronidase (U/μg prot)	1.634 ± 0.591	2.963 ± 0.412*	2.154 ± 0.732	1.534 ± 0.298△△	1.203 ± 0.372△△	1.362 ± 0.063△△
Acid phosphatase (U/μg prot)	2.684 ± 0.153	3.428 ± 0.704**	2.552 ± 2.177△△	2.637 ± 0.046△△	2.624 ± 0.127△△	2.721 ± 0.108△△

Notes: [a]Enzyme activities of β-galactosidase was expressed as μmoL ONPG/(min•mL). *P<0.05, **P<0.01 *vs*. normal group; △P<0.05, △△P<0.001 *vs*. model group

Table 3. Effects of RDN and Indomethacin on Enzymic Antioxidant Status in MSU Crystal-Induced GA Rats (n=10, $\bar{x} \pm$ SEM)

Parameter	Normal group	Model group	RDN group (100 mg/kg)	RDN group (300 mg/kg)	RDN group (900 mg/kg)	Indometacin group (3 mg/kg)
Serum						
Gpx (U)	11.998 ± 4.340	4.721 ± 0.896*	9.905 ± 1.446△△	11.194 ± 2.257△△	11.967 ± 1.957△△	11.985 ± 1.157△△
SOD (U/mL)	153.142 ± 17.532	110.110 ± 21.795*	214.079 ± 12.376△△	141.175 ± 13.388	183.118 ± 15.482△	129.972 ± 10.169
MDA (nmol/mL)	5.791 ± 0.522	8.509 ± 0.551*	7.124 ± 0.463	6.085 ± 0.495△△	5.913 ± 0.512△△	4.523 ± 0.307△△
Liver						
Gpx (U)	281 .626 ± 47.949	70.745 ± 41.415**	147.811 ± 9.716△△	211.668 ± 24.603△△	219.676 ± 65.207△△	688.209 ± 35.602△△
SOD (U/mg prot)	214.092 ± 25.611	155.507 ± 12.195**	168.279 ± 9.716	255.741 ± 18.315△△	187.718 ± 23.949	179.729 ± 18.254
MDA (mg prot/mL)	0.952 ± 0.153	1.368 ± 1.082*	0.857 ± 0.058	0.987 ± 0.078△	0.785 ± 0.058△△	0.762 ± 0.286△△
Kidney						
Gpx (U)	254.536 ± 57.713	67.978 ± 18.286*	64.221 ± 17.425	71.045 ± 22.841	99.729 ± 10.965△△	100.106 ± 21.825△
SOD (U/mg prot)	171.529 ± 21.515	114.743 ± 13.217*	368.671 ± 21.486△△	163.018 ± 9.871	207.793 ± 17.995	178.243 ± 19.435
MDA (mg prot/mL)	1.121 ± 0.062	1.518 ± 0.119*	1.042 ± 0.052△△	0.768 ± 0.079△△	0.961 ± 0.816△△	0.752 ± 0.307△△

Notes: Gpx: glutathione peroxidase; SOD: superoxide dismutase; MDA: malonaldehyde. *P<0.05, **P<0.01 *vs*. normal group; △P<0.05, △△P<0.01 *vs*. model group

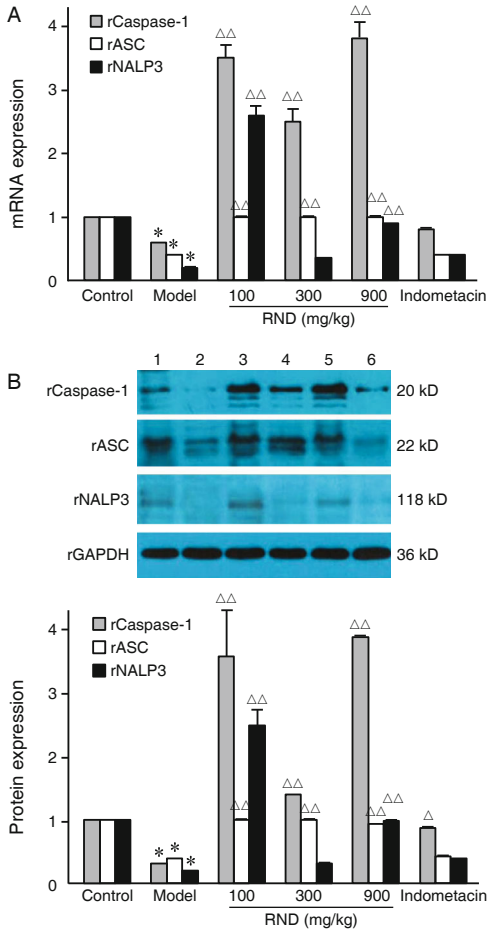

Figure 2. Effects of RDN and Indomethacin on mRNA (A) and Protein Expression (B) of NALP3 Inflammasome Components in MSU Crystal-Induced GA Rats (n=6, x ± SEM)

Notes: 1: control; 2: model; 3, 4, 5: RND 100, 300, 900 mg/kg, respectively; 6: indometacin. $^{*}P$<0.01 *vs*. control group; $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01 *vs*. model group

the mononuclear cells in the model group were both decreased (P<0.01). RDN at the high, middle and low doses significantly increased the mRNA (P<0.01) and protein expression (P<0.01) of ASC. Indomethacin also increased the mRNA and protein expression of ASC. However, these effects were not statistically significant.

Compared with the normal group, the levels of mRNA and protein expression of NALP3 in the serum of the mononuclear cells in the model group were both decreased (P<0.001). RDN at the high and low doses both increased the mRNA (P<0.01) and protein expression (P<0.01) of NALP3. Indomethacin also increased the mRNA and protein expression of NALP3. However, the effects were not statistically significant.

DISCUSSION

RDN is commonly used to treat GA and hyperuricemia in Chinese medicine. However, the exact mechanism of its efficacy is unclear. In the present study, we first demonstrated that RDN significantly reduced the activities of the lysosomal enzymes and regulated the antioxidant abilities of the organism including increasing the activities of glutathione peroxidase and total superoxide dismutase and decreasing the levels of malondialdehyde. RDN significantly reduced the contents of inflammatory factors as well. Furthermore, RDN increased both the mRNA and protein levels of caspase-1, ASC and NALP3 in the mononuclear cells of the peripheral blood of the GA rats. These findings confirm that RDN affects GA by regulating the inflammasome, which plays an important role in the disease.[21]

Hyperuricemia is the most important risk factor for GA. A concentration of the uric acid in the blood above 416 μmol/L is defined as hyperuricemia. However, the possibility of a GA attack cannot be excluded when the concentration is below 416 μmol/L. Although approximately 10% of the patients with hyperuricemia will develop GA, approximately 80%–90% of the patients with GA suffer from hyperuricemia during an attack.[22] Our previous studies focused on the urate transporters to evaluate the mechanism by which RDN reduced the concentration of uric acid, whereas the present study aimed to discover the mechanism of RDN from its anti-inflammatory properties.[23] The most obvious feature of a GA attack is the neutrophil accumulation in the joint fluid. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytose monosodium urate crystals and release mediators to amplify the inflammatory reaction.[24] In the present study, lysosomal enzymes were found to be increased in monosodium crystal-induced rats. Lysosomes and lysosomal enzymes play vital roles in tissue injury, repair, inflammation and phagocytosis. They participate in pathologic processes such as inflammation, degeneration, cancer and rheumatoid arthritis. It has been further postulated that lysosomal enzymes are released in order to stimulate the synthesis of prostaglandins.[25] To summarize, extracellular release of lysosomal enzymes may be crucial to the pathogenesis of tissue injury and inflammation.[26] In this study, it was found that the activities of lysosomal enzymes were all increased in the livers, kidneys and blood of the model group. RDN regulated these activities toward normal levels. However, this effect

did not show dose-dependence.

Free radicals have also been reported to be involved in producing damage to the arthritic joint. Considerable evidence suggests that oxidative stress and reactive oxygen species play significant roles in several aspects of acute and chronic inflammation.[27] Our study found that the level of lipid peroxidation in the MSU-induced GA rats was increased, suggesting that oxygen free radicals play an important role in the phagocytosis of the immune complexes in GA. Under normal condition, oxygen free radicals and lipid peroxidation are coordinated and in dynamic balance. Disturbances in this coordination and dynamic balance will lead to a series of metabolic abnormalities and oxygen free radical chain reactions that can harm the bio-membranes and their functions. Furthermore, these alterations will lead to pathological changes in the cells, fibrillation, and large area of cell damage and damage to the nerves, tissues and organs. The prevention of lipid peroxidation is an essential process in all the aerobic organisms because lipid peroxidation products can cause DNA damage.[28]

Our study found that the activities of both Gpx and SOD were decreased in MSU-induced GA rats, whereas the levels of MDA were increased. RDN restored the levels of these indicators to the normal conditions, indicating that RDN may treat GA by regulating the anti-oxidative capacity of the body. In this study, the levels of TNF-α, IL-1β and IL-8 were all increased in GA rats. The consequences of the increased release of these inflammatory cytokines, partially due to the stimulation of oxidative stress,[29] include cytokine imbalance, immune dysfunction and even liver cell apoptosis. RDN regulated the concentrations of these inflammatory cytokines to the normal level, indicating that RDN may regulate the generation of inflammatory factors by influencing the inflammasome or other signal pathways that are involved in inflammation.

In the process of a GA attack, NALP3, composed of NALP3, caspase-1 and ASC, plays an important role. During a GA attack, mononuclear macrophages and other immune cells ingest the MSU. This reaction leads to changes in both the structure and the assembly of the inflammasome. In this study, the expressions of NALP3, caspase-1 and ASC at both the mRNA and protein levels in the GA-model rats were decreased, indicating that there may be negative regulators that restricted the expressions of the NALP3 inflammasome. The decreased expressions of NALP3 inflammasome further restricted the activation of caspase-1. The production of the mature forms of IL-1β, TNFα and IL-8 also decreased. RDN significantly regulated the mRNA and protein expressions of NALP3, caspase-1 and ASC to normal levels. However, at the middle (300 mg/kg) dose of RDN, there were no significant effects on the mRNA and protein expressions of NALP3 compared with the model group. In other words, the high (900 mg/kg) and low (100 mg/kg) doses of RDN were more effective than at the middle dose (300 mg/kg), and thus, the effects of RDN were not dose-dependent. It should be noted that these treatments were given prophylactically in this study. It is possible that RDN protected the rats so that the degree of the symptoms of GA was alleviated, and the extent of the self-limiting responses was decreased. A probable explanation might be that the middle dose has a better protective effect, while the low and high doses have a better treatment effect.

In parallel with the effects of RDN, the positive control drug indomethacin also treated GA by influencing the NALP3 inflammasome. However, only the expression of the caspase-1 protein in this study was affected by indomethacin. In future studies, the effects of RDN on the expressions of the elements of the "TLR2/4-IL1R receptor" signal pathway will be studied to investigate whether RDN affects the expressions of IL-1β etc.

Conflict of Interest

The authors have no financial conflict of interest.

Author Contributions

Zhou Q and Zhang N carried out the experiments, Yu DH contributed to statistical analysis, Zhou Q wrote the manuscript, Lu SM designed the project and supervised the work and provided the facilities for the study. All authors read the manuscript and approved the final version.

Acknowledgments

The first author of this paper is currently working at the postdoctoral research station of Heilongjiang University of Chinese Medicine.

Abbreviation: NALP3: NACHT (neuronal apoptosis inhibitor protein, class 2 transcription activator, heterokaryon incompatibility,

and telomerase-associated protein 1), LRR (leucine-rich repeat) and PYD (pyrin domain) domains-containing protein 3.

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