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Curcumin attenuates murine lupus via inhibiting NLRP3 inflammasome

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ABSTRACT

Despite rapid progress in the understanding of systemic lupus erythematosus (SLE), there is still an urgent need for novel and more effective interventions. Curcumin, a natural polyphenol compound, has been shown to be anti-inflammatory in various disorders. In this study, we investigated the potential therapeutic value of curcumin in SLE. Lupus-prone female MRL/lpr mice were treated with curcumin. The development and extent of nephritis were assessed by monitoring proteinuria and by histologic analysis. Serum anti-dsDNA levels were measured by enzyme-linked immunosorbent assay. Kidney samples were analyzed by Western blot. In vitro, mouse podocytes were used for investigation in the presence of mouse anti-dsDNA antibody-positive (anti-dsDNA+) serum. Curcumin treatment dramatically decreased proteinuria and renal inflammation. Serum anti-dsDNA levels and spleen size were also reduced by curcumin. In addition, curcumin reduced NLRP3 inflammasome activation in lupus-prone mice. In vitro, curcumin significantly inhibited anti-dsDNA+ serum induced expression of NLRP3 inflammasome in podocytes. Overall, these data demonstrate the potential use of curcumin in SLE treatment.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by generation of numerous autoantibodies. Lupus nephritis (LN) is a severe manifestation of SLE with a significant prognostic impact. Over the last decades, despite improved life expectancy and life quality for SLE patients due to advances in treatment, treatment of this disease remains challenging [1].

Inflammation-related signaling pathways contribute to tissue injury and clinical presentations of SLE. NLRP3 inflammasome is a key player in LN. Circulating antigen-antibody complexes can activate the NLRP3 inflammasome [2,3]. Increased formation of neutrophil extracellular traps (NETs) can activate the NLRP3 inflammasome in human lupus-derived macrophages, resulting in enhanced production of inflammatory cytokines [4]. Previous data reveal that NLRP3 inflammasome promotes LN in several lupus-prone models [5–7]. Kahlenberg and colleagues show that Caspase-1, the central component of NLRP3 inflammasome, is essential for type I interferon responses, autoantibody production and renal damage in the pristine-induced lupus [8]. Lu et al. demonstrates that over-activated NLRP3 inflammasome in myeloid cells leads to severe organ damage in experimental lupus [9]. Podocytes, localized on the outer basement membrane, participate actively in the development of glomerular injury mediated by immune cells. Recent evidence shows that NLRP3 inflammasomes were activated in podocytes from lupus-prone mice and from LN patients, which contributes to podocytes injuries and proteinuria in LN [10].

Turmeric, extracted from the powdered dry rhizome of *Curcuma longa*, is traditionally used as a spice and coloring in foods. Curcumin is a main natural ingredient of turmeric and has been used for long in indigenous medicine and is approved as safe by the Food and Drug Administration of USA [11,12]. Numerous studies have shown the bioactivity and health benefits of curcumin, including anti-inflammatory, antioxidant, and antitumor effects [13]. Increasing evidence suggests that curcumin can offer protection against inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel disease [14,15]. Interestingly, recent findings reveal a mechanism through which curcumin represses inflammation via regulating NLRP3 [16]. Effects of curcumin on lupus-prone mice have been reported by several investigators [17–19] and one clinical trial demonstrated that curcumin reduced proteinuria in LN patient [20]. However, the therapeutic mechanism of curcumin on renal inflammation in SLE remains to be investigated. In this study, we applied a widely used mouse model of SLE

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to investigate the effect and therapeutic mechanism of curcumin on murine LN.

2. Materials and methods

2.1. Mice

The animal experiment in the present study was approved by the Ethics Committee of First Affiliated Hospital, Sun Yat-sen University. Female MRL/lpr mice were purchased from the SLAC Laboratory (Shanghai, China) and maintained in the Sun Yat-sen University under specific pathogen-free conditions.

2.2. Curcumin administration

Curcumin (Selleck Chemicals, USA) was suspended in corn oil (vehicle) and MRL/lpr mice received curcumin (200 mg/kg) or an equal volume of vehicle (n = 12 mice/group) by daily gavage administration, starting from 12 weeks of age for 8 weeks.

2.3. Mouse podocytes and treatment

Conditionally immortalized mouse podocytes were obtained from Shanghai Ruilu Technology Co. (Cat. no FDCC-MSN059, Shanghai, China). To induce proliferation, the cells were grown on type I collagen-coated plastic culture bottles (BD Biosciences, USA) at 33 °C in RPMI-1640 culture medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin mixture (Sigma, USA), with added recombinant mouse IFN-γ (20 U/ml, Sigma, USA). To induce phenotype differentiation, the podocytes were grown at 37 °C and deprived of γ-interferon (growth-restrictive conditions) in culture for 10–14 days for the following experiments.

Podocytes were incubated in the medium and stimulated with anti-dsDNA + serum from diseased mice or control serum from healthy mice (5% final concentration) for 24 h.

2.4. Evaluation of urine protein

The level of urine albumin was assessed by ELISA (Bethyl Laboratories Inc., USA) and urine creatinine was measured using Creatinine Colorimetric Assay Kit (Cayman chemical, USA) every two weeks according to the manufacturer's instructions. The urine albumin-to-creatinine ratio was expressed as μg/mg.

2.5. Assessment of nephritis

Sections in paraffin were processed with Hematoxylin and Eosin (H &E) staining. The glomerular lesions were scored on a scale of 0–3 and the mean score was calculated from the sum of the scores for 40 random glomeruli per kidney [7].

2.6. ELISA

Serum anti-dsDNA and IL-1β were detected using enzyme-linked immunosorbent assay (ELISA) according to our previous publication [7]. Normal mouse IgG was used as negative control. Cytokines were determined using ELISA kits (R&D Systems) according to the manufacturer's instructions.

2.7. Immunoblotting

The kidney tissues or cultured cells were homogenized in cell lysis buffer (Cell Signaling Technology, USA) and then centrifuged at 15,000*g* at 4 °C for 30 min. For the immunoblot analysis, proteins were subjected to electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gel, and transferred to nitrocellulose membrane (Millipore, USA). Membranes were incubated with primary antibodies: anti-NLRP3 (Adipogen, USA), anti-caspase1p20 (Cell Signaling Technology, USA) and anti-GAPDH (Cell Signaling Technology, USA). Membranes were washed and incubated with secondary antibody conjugated to horseradish peroxidase (Cell Signaling Technology) at room temperature for 1 h. These proteins were detected with horseradish peroxidase-conjugated secondary antibodies. The protein bands were visualized with an enhanced chemiluminescence detection kit (Millipore, USA).

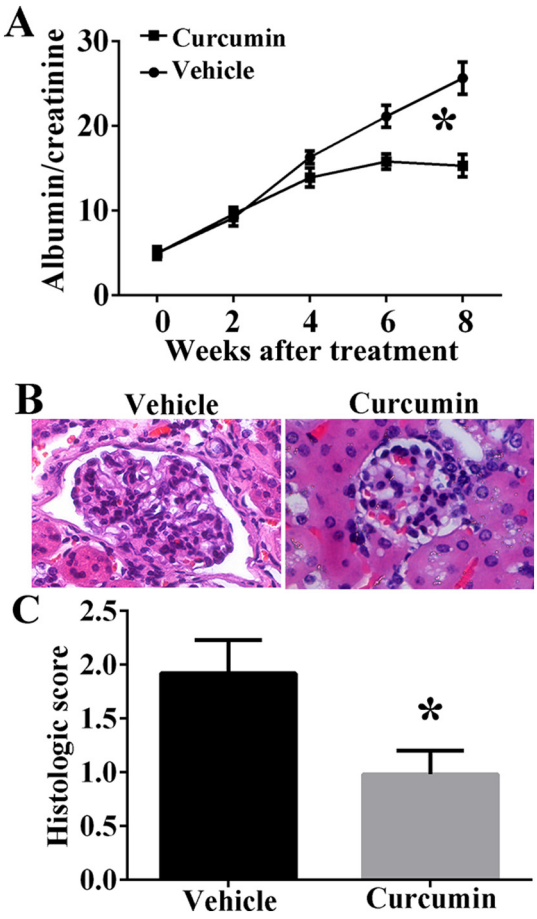

Fig. 1. Curcumin attenuated renal lesions in MRL/lpr mice. After treatment with curcumin for 8 weeks, proteinuria and histological parameters were measured. A. The urine albumin/creatinine ratio was expressed as μg/mg. B. HE staining of kidney sections showed significantly decreased inflammatory cell infiltration in glomeruli in curcumin-treated group (Magnification: ×200). C. Histologic damage index of kidneys in MRL/lpr mice. Values are presented as mean ± SEM. *p < 0.05 by Student *t*-test versus the vehicle-treated group; n = 12 mice per group.

2.8. Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using Student *t*-test or one-way ANOVA followed by Dunnett's posttest. All data were processed by SPSS 19.0 software. $p < 0.05$ was considered statistically significant.

3. Results

Curcumin treatment decreased proteinuria and renal inflammation.

First, we tested whether curcumin has therapeutic value in murine SLE. Urine protein secretion was assessed every other week. We detected the albumin/creatinine ratio. Compared with vehicle-treated

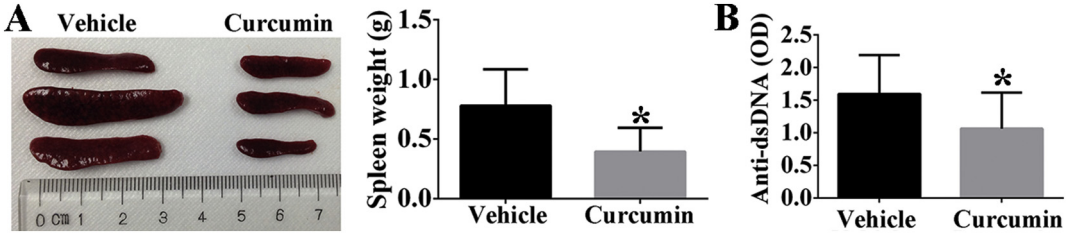

Fig. 2. Curcumin reduced enlargement of the spleen and serum anti-dsDNA antibody in the MRL/lpr mice. A. In MRL/lpr mice, the organ weights of the spleen were significantly reduced by curcumin treatment. B. Serum anti-dsDNA antibody was significantly decreased in curcumin-treated mice. Values are presented as mean ± SEM. *p < 0.05 by Student *t*-test versus the vehicle-treated group; n = 12 mice per group.

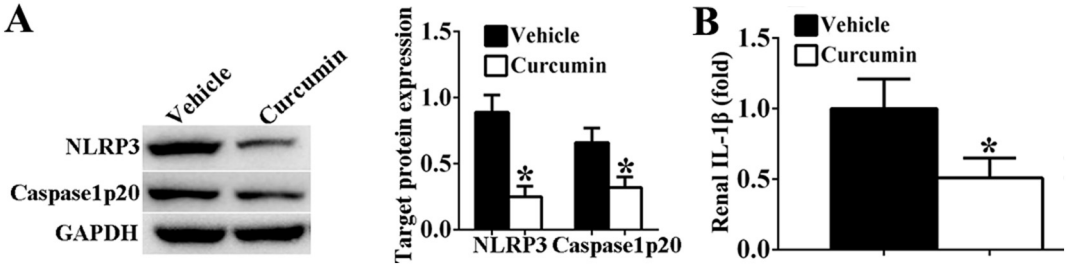

Fig. 3. Curcumin reduced NLRP3 inflammasome in the kidneys of MRL/lpr mice. MRL/lpr mice were treated for 8 weeks and NLRP3 inflammasome activation was examined by western blot analysis. A: The presented blots were representatives of those obtained from 12 mice per group. Semi-quantitative analysis showed that NLRP3 and Caspase-1p20 expressions were significantly inhibited after 8 weeks treatment with curcumin compared to vehicle-treated group. B. Renal IL-1β levels were measured by ELISA. Results are shown as fold change of control group. *p < 0.05 by Student *t*-test versus the vehicle-treated group.

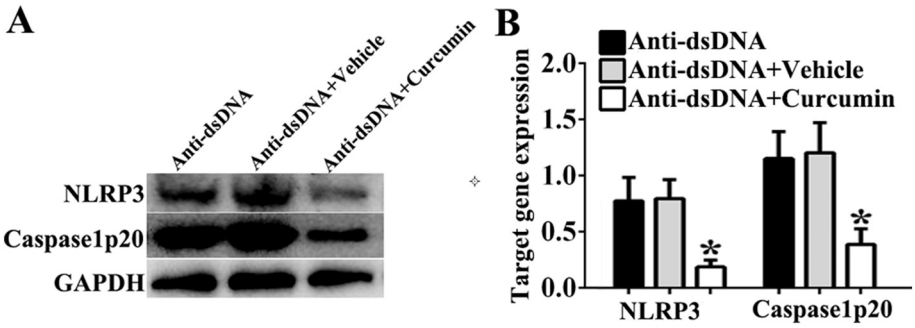

Fig. 4. Curcumin attenuates anti-dsDNA-induced NLRP3 inflammasome activation in podocytes. Podocytes were incubated in the medium and stimulated with anti-dsDNA + serum from diseased mice or control serum from healthy mice (5% final concentration) for 24 h. Data are mean ± SEM from three independent experiments. *p < 0.05 by one-way ANOVA followed by Dunnett's posttest vs vehicle-treated group.

mice, curcumin treatment significantly decreased the proteinuria ($p < 0.05$, Fig. 1A). Furthermore, at the end of the study, mouse kidneys were collected and subjected to hematoxylin and eosin (H&E) staining. As shown in Fig. 1B, vehicle-treated animals developed enlarged hypercellular glomeruli with inflammatory infiltrates. In contrast, curcumin treatment significantly attenuated nephritis as indicated by the semi-quantitative analysis (Fig. 1C).

Curcumin reduced spleen enlargement and serum anti-dsDNA levels.

To investigate the inhibitory effects for splenomegaly as manifestations of SLE, the weights of the spleen were measured after treatment in MRL/lpr mice. Spleen weights were diminished ($p < 0.05$, Fig. 2A) in curcumin-treated mice. Compared with vehicle treatment, serum anti-dsDNA level was significantly inhibited ($p < 0.05$, Fig. 2B).

Curcumin reduced NLRP3 inflammasome in lupus-prone mice.

NLRP3 expression was significantly reduced after treatment with curcumin compared to vehicle-treated mice (Fig. 3A). The expression of caspase1p20, active form of caspase-1 (Fig. 3A), and the level of renal IL-1β, downstream of NLRP3 activation, were significantly decreased after curcumin treatment (Fig. 3B).

Curcumin attenuates anti-dsDNA-induced NLRP3 inflammasome activation in podocytes.

Anti-dsDNA can activate NLRP3 inflammasome. Furthermore, we found that curcumin could affect anti-dsDNA-induced NLRP3 inflammasome activation, as demonstrated by reduced expression of NLRP3 and caspase1p20 in podocytes (Fig. 4A and B).

4. Discussion

Despite considerable progress in treatment modalities, SLE remains a great challenge for clinicians because patients are often refractory to conventional treatments, highlighting the need to develop new and effective therapies. In this study, we employed the well-established murine model of SLE, the MRL/lpr mice to evaluate curcumin as a therapeutic agent. Results show that curcumin treatment attenuated nephritis and anti-dsDNA antibody production in MRL/lpr mice. Mechanistically, this effect may occur through suppression of NLRP3 inflammasome. We further show that curcumin in vitro inhibited NLRP3 inflammasome activation induced by human anti-dsDNA antibody in podocytes. Thus, these results suggest that curcumin may be a promising agent for the treatment of human SLE.

Although curcumin has been extensively used in traditional medicine in clinical practice for the treatment of rheumatoid arthritis, ulcerative colitis, arteriosclerosis, skin lesions and chronic asthma [21–25], its anti-inflammatory mechanism has not been fully understood. Curcumin protects against diabetes-associated abnormalities in the kidneys by changing post-translational modifications of histone H3, expression of HSP-27, MAP kinase p38 and inhibiting p300 and nuclear

factor-κB [26,27]. Curcumin treatment decreased the ROS level in blood serum of chronic asthmatic mice and significantly decreased the phosphorylation of JNK, ERK1/2, and p38 and COX-2 expression thereby nuclear factor κB (NF-κB) activation, suggesting that curcumin protects against asthma via action on mitogen-activated protein kinase (MAPK)/NF-κB signaling pathways [25]. Curcumin regulated serum alanine transaminase (ALT), aspartate transaminase (AST) and inhibited activation of the mitogen-activated protein kinases/c-Jun NH2-terminal kinase (P38/JNK) cascade in the livers of LPS-induced rats [28].

Recent studies have suggested that the inhibition of NLRP3 may be responsible for the anti-inflammatory action of curcumin. Curcumin suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock [29]. Curcumin inhibited NLRP3 inflammasome through suppressing TLR4/MyD88/NF-κB and P2X7R pathways in PMA-induced macrophages [30]. Of interest, curcumin suppresses osteoarthritis, monosodium urate crystal-induced peritoneal inflammation and dextran sulfate sodium (DSS)-induced colitis through inhibiting NLRP3 inflammasome activation and IL-1β production in vivo [16,31,32]. Consistent with previous studies, in this study, we found that curcumin inhibited NLRP3 inflammasome/IL-1β in vivo in lupus-prone mice and in vitro in anti-dsDNA + serum-induced podocytes. One of the hallmarks of LN is the development of proteinuria, which is usually followed by a progressive decline in renal function. Moreover, our data showed that podocyte activation of NLRP3 inflammasomes contributes to the development of proteinuria in lupus nephritis [10]. These findings indicate that curcumin inhibited disease progression in lupus-prone mice, which is at least partly dependent on inhibition of NLRP3 inflammasomes.

Taken together, curcumin exerted a protective effect against LN in MRL/lpr mice. We speculate that the protective effects of curcumin in LN may involve, at least in part, its inhibition of NLRP3 inflammasome.

Conflict of interest

All authors declare no conflicts of interest in this study.

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