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•Original article•

10,11-Dehydrocurvularin attenuates inflammation by suppressing NLRP3 inflammasome activation

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[ABSTRACT] 10,11-Dehydrocurvularin (DCV) is a natural-product macrolide that has been shown to exert anti-inflammatory activity. However, the underlying mechanism of its anti-inflammatory activity remains poorly understood. Aberrant activation of the NLRP3 inflammasome is involved in diverse inflammation-related diseases, which should be controlled. The results showed that DCV specifically inhibited the activation of the NLRP3 inflammasome in association with reduced IL-1β secretion and caspase-1 activation, without effect on the NLRC4 and AIM2 inflammasomes. Furthermore, DCV disturbed the interaction between NEK7 and NLRP3, resulting in the inhibition of NLRP3 inflammasome activation. The C=C double bond of DCV was required for the NLRP3 inflammasome inhibition induced by DCV. Importantly, DCV ameliorated inflammation *in vivo* through inhibiting the NLRP3 inflammasome. Taken together, our study reveals a novel mechanism by which DCV suppresses inflammation, which indicates the potential role of DCV in NLRP3 inflammasome-driven inflammatory disorders.

[KEY WORDS] 10,11-Dehydrocurvularin; Inflammation; NLRP3 inflammasome; NEK7-NLRP3 interaction
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Introduction

The NLRP3 inflammasome is a cytosolic protein complex consisting of the innate immune receptors NLRP3, ASC, and caspase-1 [1, 2]. Danger- and pathogen-associated molecular patterns can drive NLRP3 inflammasome assembly and activation, leading to the release of proinflammatory cytokines IL-1β and IL-18 [3-6]. K^+ efflux, elevated ROS levels, mitochondrial damage, and lysosomal rupture are associated with NLRP3 inflammasome activation [7-9]. Hyperactivation of the NLRP3 inflammasome contributes to several inflammatory disorders, such as atherosclerosis, Alzheimer's disease, colitis, sepsis and type 2 diabetes (T2D), implying that NLRP3 inflammasome inhibition is required to achieve good control of these diseases [10-15]. Thus, the NLPR3 inflammasome represents a potential target for inflammatory diseases [16-18]. Recently, a few small-molecule compounds, including β-hydroxybutyrate, MCC950, oridonin, oroxindin, pristimerin, tranilast, and mangiferin, have been demonstrated to exert anti-inflammatory activity through inhibiting the NLRP3 inflammasome *in vitro* and *in vivo* [19-26]. Therefore, the discovery of novel NLPR3 inflammasome inhibitors is of clinical importance for NLRP3-driven diseases.

10,11-Dehydrocurvularin (DCV), a natural benzenediol lactone, is the secondary metabolite of many fungi [27]. Other reports and our previous studies showed that DCV exhibits a wide range of anticancer activities, including cell cycle arrest, angiogenesis and invasion suppression [28-30]. In addition to in-

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These authors have no conflict of interest to declare.

ducing cytotoxicity against cancer cells, DCV exerted antioxidant and anti-inflammatory activity [31, 32]. Our recent study has also demonstrated that DCV inhibits the release of proinflammatory cytokines and inflammatory response by suppressing the NF-κB or MAPK signaling pathway [33]. Moreover, DCV exerted a significant protective role in sepsis and colitis [32, 34].

Herein, we demonstrated that DCV specifically inhibited NLRP3 inflammasome activation to exert anti-inflammatory effect. DCV suppressed NLRP3 inflammasome activation *via* its α, β-unsaturated carbonyl group and then blocked the NLRP3-NEK7 interaction, which is critical for NLRP3 inflammasome assembly and activation. Interestingly, DCV ameliorated NLRP3-dependent inflammation in a mouse model. These findings suggest that DCV is a potential inhibitor of NLRP3 inflammasome activation for the treatment of NLRP3-driven diseases.

Materials and Methods

Animals

All mice were housed under specific pathogen-free (SPF) conditions in an air-conditioned room. *Nlrp3*$^{-/-}$ mice were kept as previoiusly described [22]. All the mice were bred on a C57BL/6J background. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Hubei University of Medicine in China (approval number: 2020-047).

Reagents

DCV and R-DCV were prepared as previously described [30]. Adenosine triphosphate (ATP, A2383), lipopolysaccharide (LPS, L2880), monosodium urate (MSU, U2875) crystals, nigericin (N7143), anti-Flag (F2555) and anti-VSV (V4888) antibody were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anti-NEK7 (sc-50756), anti-ASC (sc-22514), anti-human IL-1β (sc-7884) and anti-caspase-1 (sc-515) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The anti-mouse caspase-1 (AG-20B-0042) antibody was supplied by AdipoGen (San Diego, CA, USA). Anti-mouse IL-1β (AF-401-NA) was obtained from R&D Systems (Minneapolis, MN, USA).

Cell preparation and stimulation

Bone marrow macrophages (BMDMs) were isolated from bone marrow cells and cultured in RPMI 1640 complete medium (31800022, Thermo Fisher, Waltham, MA, USA) containing macrophage colony-stimulating factor (M-CSF, 50 ng·mL^{-1}). Bone marrow dendritic cells (BMDCs) were derived from bone marrow cells in RPMI 1640 complete medium supplemented with granulocyte-macrophage colony stimulating factor (GM-CSF, 50 ng·mL^{-1}). M-CSF (AF-315-02) and GM-CSF (AF-300-03) were obtained from Peprotech (Rocky Hill, NJ, USA).

NLRP3 inflammasome activation

To activate NLRP3 inflammasomes, 1 × 10^6 cells were primed with LPS (200 ng·mL^{-1}) and then stimulated with nigericin, ATP or MSU. BMDMs were infected with *Salmonella typhimurium* to activate NLRC4 inflammasomes. Poly (dA:dT) (Thermo Fisher, Waltham, MA, USA) was transfected for AIM2 inflammasome activation. The cells were treated with DCV for 30 min after LPS stimulation.

Enzyme-linked immunosorbent assay (ELISA)

Supernatants were obtained from cell culture, and the levels of serum IL-1β (88-7013-88), IL-18 (BMS618/3) and TNF-α (88-7324-88) were measured according to the manufacturer's instructions (eBioscience, San Diego, CA, USA).

Transfection and co-immunoprecipitation

For endogenous interactions, LPS-primed BMDMs with or without DCV were stimulated and lysed. Then, proteins were immunoprecipitated with the indicated antibodies. For exogenous interaction, plasmids were transfected into HEK-293T cells using Lipofectamine 3000 (Thermo Fisher, Waltham, MA, USA). After DCV treatment, the cells were lysed, and the proteins were immunoprecipitated with a Flag-conjugated bead antibody.

LPS-induced systemic inflammation in vivo

Adult male C57BL/6J mice (n = 6) were intraperitoneally injected with LPS (20 mg·kg^{-1}) with or without DCV (10 mg·kg^{-1}). After 4 h, the peritoneal fluid and blood were collected. The levels of IL-1β, IL-18, and TNF-α in the serum and peritoneal fluid were measured by ELISA.

MSU-induced peritonitis in vivo

Adult male C57BL/6J mice (n = 6) were intraperitoneally injected with DCV (10 mg·kg^{-1}) before injection of MSU (0.5 mg of MSU per mouse). After 6 h, the peritoneal cavities and blood were collected. Neutrophil counts were measured, and the levels of IL-1β in serum and peritoneal lavage fluid were measured using ELISA.

Statistical analysis

Data are shown as the mean ± SD. Statistical analysis was conducted using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). P values < 0.05 indicated significance.

Results

DCV inhibits NLRP3 inflammasome activation

To evaluate the inhibitory effect of DCV on NLRP3 inflammasome activation, we first detected whether DCV suppressed caspase-1 cleavage and IL-1β secretion in LPS-primed BMDMs (Fig. 1A). The results indicated that DCV suppressed caspase-1 activation and IL-1β secretion in a dose-dependent manner (Figs. 1B and 1C). DCV also inhibited nigericin-induced IL-18 secretion (Fig. 1D). However, DCV did not affect the secretion of TNF-α, an inflammasome-independent cytokine (Fig. 1E). In addition, DCV treatment did not affect cell growth (Fig. 1F), suggesting that the effect of DCV on IL-1β and IL-18 release is not attribuable to its toxicity. Similarily, DCV inhibited caspase-1 activation and IL-1β secretion in LPS-primed mouse peritoneal macrophages (Figs. 1G and 1H) and BMDCs (Supplementary Figs. 1A and

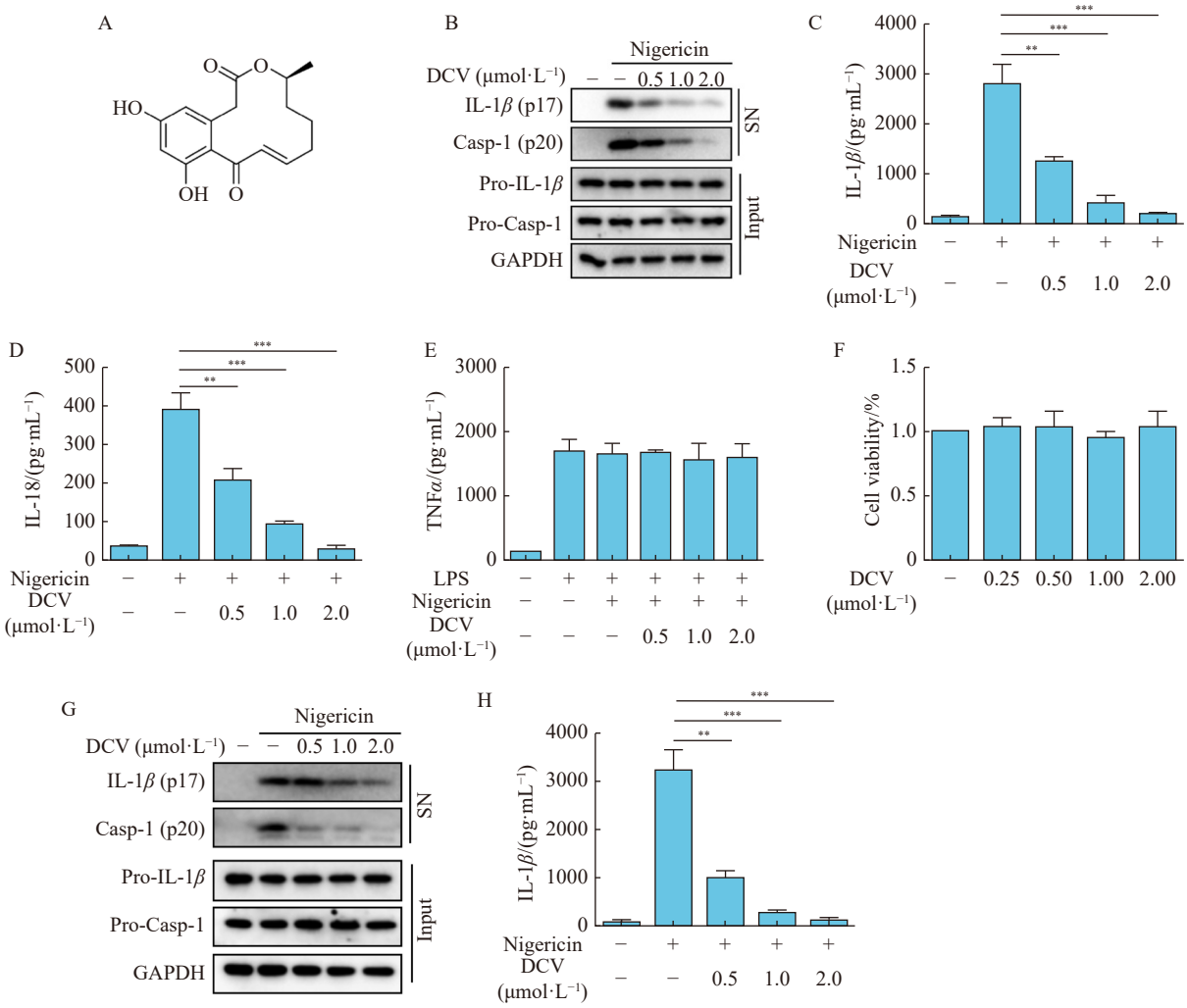

Fig. 1 DCV inhibits NLRP3 inflammasome activation. (A) The structure of DCV. (B) LPS-primed BMDMs were treated with DCV (0.5, 1.0 or 2.0 μmol·L^{-1}) for 30 min and then stimulated with nigericin for 30 min. Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in culture supernatants (SN) and pro-IL-1*β* and pro-caspase-1 (p45) in whole cell lysates (Input). (C−E) Production of IL-1*β* (C), IL-18 (D) and TNF-*α* (E) in SN from the LPS-primed BMDMs described in (B). (F) LPS-primed BMDMs were treated with the indicated concentrations of DCV for 24 h. Then, cell growth was analyzed. (G) LPS-primed mouse peritoneal macrophages were treated with DCV (0.5, 1.0 or 2.0 μmol·L^{-1}) and then stimulated with nigericin. Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (H) Production of IL-1*β* in SN from the LPS-primed mouse peritoneal macrophages described in (G). Data are expressed as the means ± SD of at least three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control

1B). These results suggested that DCV inhibited NLRP3 inflammasome activation in cells.

DCV specifically suppresses NLRP3 inflammasome activation

To further examine whether DCV specifically suppresses NLRP3 inflammasome activation, LPS-primed BMDMs were exposed to NLRP3 agonists, including nigericin, ATP and MSU with or without the presence of DCV [35]. The results showed that DCV suppressed caspase-1 activation and IL-1*β* secretion induced by all the agonists (Figs. 2A and 2B), implying that DCV is a broad NLRP3 inflammasome inhibitor. Then, we investigated the effects of DCV on NLRC4 and AIM2 inflammasome activation. The results showed that DCV exerted a minimal effect on *Salmonella typhimurium* infection-induced NLRC4 inflammasome activation (Figs. 2C and 2D) and poly(dA:dT) transfection-induced AIM2 inflammasome activation (Figs. 2E and 2F). These findings indicated that DCV specifically inhibited NLRP3 inflammasome activation but did not affect the NLRC4 and AIM2 inflammasomes.

DCV inhibits NLRP3 inflammasome assembly

Next, we investigated the mechanism by which DCV inhibited NLRP3 inflammasome activation. The results showed that DCV did not suppress nigericin-induced ROS production (Supplementary Fig. 2), which is the upstream signaling event of NLRP3 activation [36]. We thus evaluated the effect of DCV on NLRP3 inflammasome formation. The NEK7 and NLRP3 interaction is a critical process for NLRP3 oligomer-

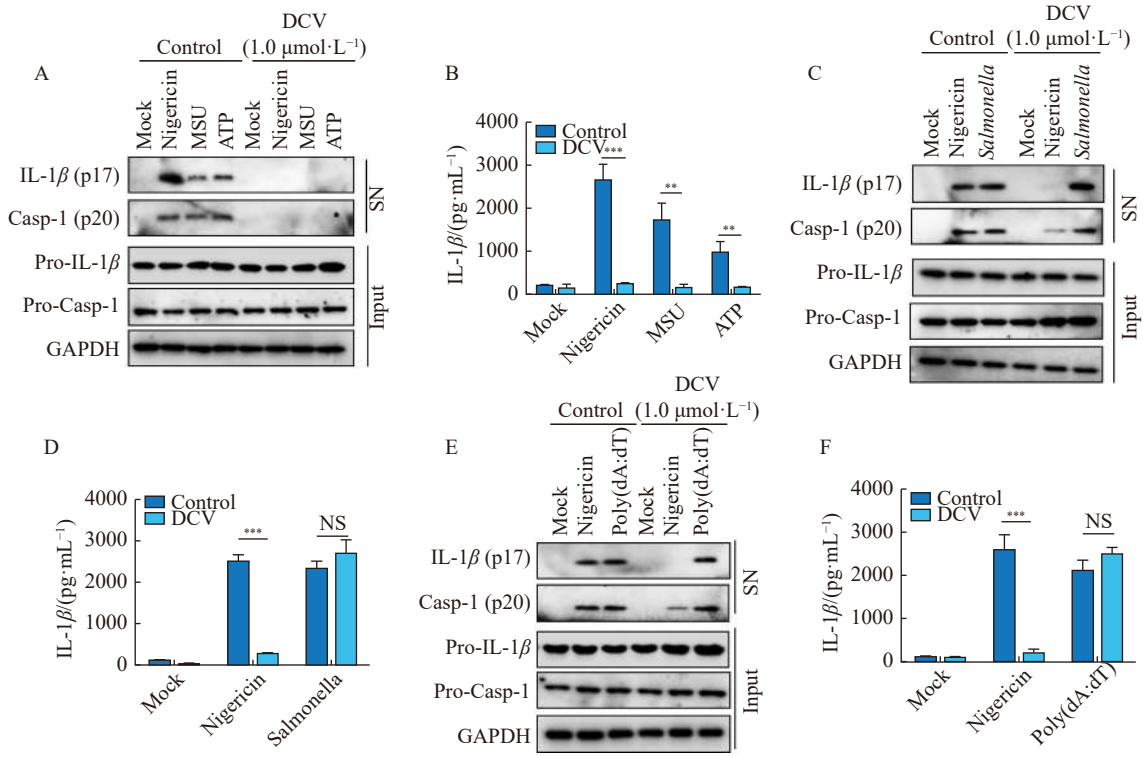

Fig. 2 DCV specifically suppresses NLRP3 inflammasome activation. (A) LPS-primed BMDMs were treated with DCV (0.5, 1.0 or 2.0 μmol·L^{-1}) and then stimulated with MSU, nigericin or ATP. Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (B) Production of IL-1*β* in SN from the LPS-primed BMDMs described in (A). (C) LPS-primed BMDMs were treated with DCV (0.5, 1.0 or 2.0 μmol·L^{-1}) and then stimulated with nigericin or *Salmonella typhimurium* (*Salmonella*). Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (D) Production of IL-1*β* in SN from the LPS-primed BMDMs described in (C). (E) LPS-primed BMDMs were treated with DCV (0.5, 1.0 and 2.0 μmol·L^{-1}) and then stimulated with nigericin or poly(dA:dT). Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (F) Production of IL-1*β* in SN from the LPS-primed BMDMs described in (E). Data are expressed as the means ± SD of at least three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control

ization and ASC recruitment, leading to NLRP3 inflammasome assembly and activation [37, 38]. To determine whether DCV affects the formation of NLRP3 inflammasome complex, we examined the NEK7-NLRP3 and NLRP3-ASC interaction. The results indicated that DCV interrupted endogenous NEK7-NLRP3 and NLRP3-ASC interactions (Figs. 3A and 3B). Moreover, DCV blocked the interaction between NEK7 and NLRP3 in HEK-293T cells (Fig. 3C). In contrast, DCV treatment did not interrupt the NLRP3-NLRP3 interaction in HEK-293T cells (Fig. 3D). Similarly, DCV did not block the NLRP3-ASC interaction in HEK-293T cells (Fig. 3E). These observations suggested that DCV suppressed NLRP3 inflammasome activation through interrupting the NEK7-NLRP3 interaction.

DCV suppresses NLRP3 inflammasome activation in a Michael acceptor-dependent manner

We sought to determine whether the inhibitory effect of DCV on NLRP3 inflammasome activation is reversible. LPS-primed BMDMs were treated with DCV for 15 min and then washed to remove unbound drug before nigericin stimulation. The data indicated that DCV still inhibited caspase-1 activation and IL-1*β* secretion after washout (Figs. 4A and 4B), indicating an irreversible effect of DCV on the inhibition of NLRP3 inflammasome activation.

Our recent study has identified the *α*, *β*-unsaturated carbonyl group of DCV, which can serve as a Michael acceptor and play an important role in its function [30]. To determine whether this unit of DCV was important for NLRP3 inflammasome inhibition, DCV was reduced by either dithiothreitol (DTT) or glutathione (GSH). The results showed that DTT or GSH treatment prevented the inhibitory effect of DCV on NLRP3 inflammasome activation (Figs. 4C and 4D). To further determine whether the carbon-carbon (C=C) double bond of DCV was essential for NLRP3 inflammasome inactivation, we used the reduced form of DCV (R-DCV), in which the C=C double bond was reduced (Fig. 4E). R-DCV did not disrupt the interaction between NEK7 and NLRP3 under endogenous or exogenous conditions (Figs. 4F and 4G). In addition, R-DCV did not inhibit caspase-1 activation or IL-1*β* secretion (Figs. 4H and 4I). These findings suggested that DCV-induced inhibition of NLRP3 inflammasome activation is Michael acceptor dependent.

Cysteine 279 in the NACHT domain of NLRP3 is responsible for NLRP3 inflammasome activation [21]. To de-

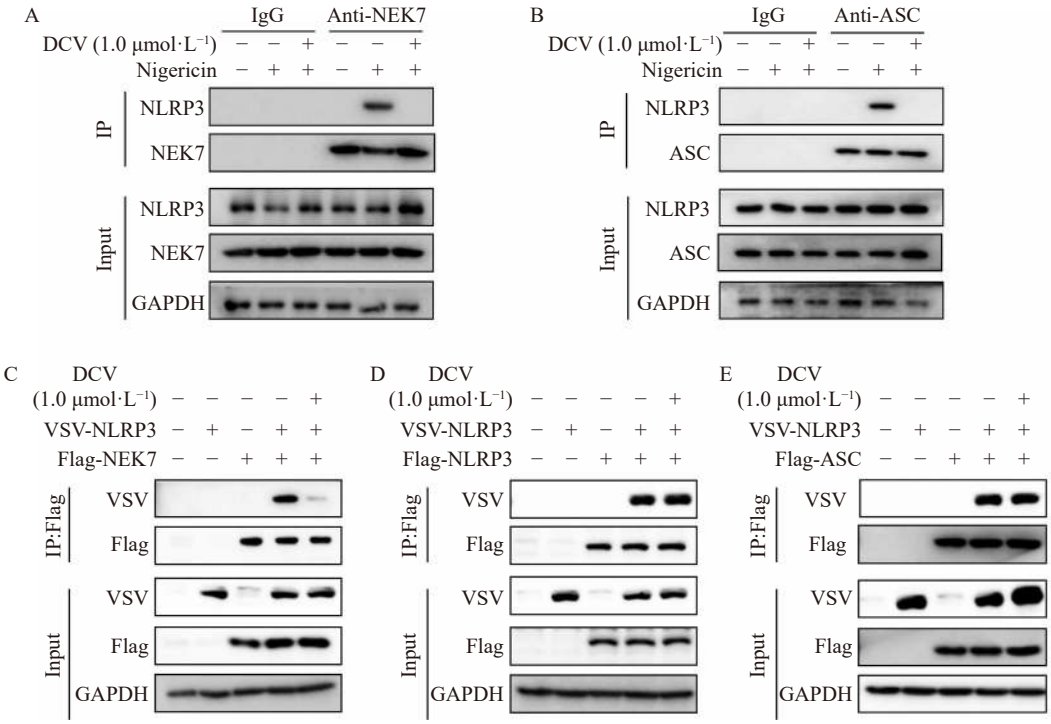

Fig. 3 DCV inhibits NLRP3 inflammasome assembly. (A) LPS-primed BMDMs were treated with DCV (1.0 μmol·L^{-1}) and stimulated with nigericin. Endogenous immunoprecipitation (IP) and Western blot analysis of the interaction of NLRP3 and NEK7. (B) LPS-primed BMDMs were treated with DCV (1.0 μmol·L^{-1}) and then stimulated with nigericin. Endogenous IP and Western blot analysis of the interaction of NLRP3 and ASC. (C) Co-IP analysis of the interaction of VSV-NLRP3 and Flag-NEK7 in HEK-293T cells. (D) Co-IP analysis of the interaction of VSV-NLRP3 and Flag-NLRP3 in HEK-293T cells. (E) Co-IP and Western blotting analysis of the interaction of VSV-NLRP3 and Flag-ASC in HEK-293T cells. Data are expressed as the means ± SD of at least three independent experiments.

termine whether the cysteine 279 of NLRP3 was critical for DCV-induced NLRP3 inflammasome inhibition, we constructed C279A NLRP3 mutants. The results showed that, although the C279A NLRP3 mutant still bound to NEK7, the interaction between NLRP3 and NEK7 was not suppressed after DCV treatment (Fig. 4J). These results suggested that cysteine 279 of NLRP3 is required for DCV-mediated NLRP3 inflammasome inactivation.

DCV ameliorates NLRP3-dependent inflammation in vivo

Since DCV inhibited NLRP3 inflammasome activation *in vitro*, in which LPS stimulated NLRP3-driven IL-1*β* expression [39], we examined the therapeutic effect of DCV in a mouse model of LPS-induced systemic inflammation. DCV markedly decreased the levels of serum IL-1*β* and IL-18 in *Nlrp3*$^{+/+}$ mice (Figs. 5A and 5B). In contrast, DCV exhibited mild effect on serum TNF-*α* levels (Fig. 5C). Consistently, IL-1*β* secretion and caspase-1 cleavage were suppressed by DCV treatment in peritoneal fluid (Fig. 5D and 5E). In addition, ASC condensed into a large cytosolic speck upon NLRP3 inflammasome activation [40], but DCV treatment suppressed ASC-speck formation (Fig. 5F). To further confirm that DCV ameliorated LPS-induced systemic inflammation dependent on NLRP3, *Nlrp3*$^{-/-}$ mice were challenged with LPS, and the therapeutic effect of DCV on systemic inflammation was absent in *Nlrp3*$^{-/-}$ mice (Figs. 5A−5E). To evaluate the safety of DCV *in vivo*, we detected its toxicity after DCV treatment. No notable differences in body weight or blood biochemical parameters were observed between the vehicle and DCV-treated groups among healthy mice (Supplementary Figs. 3A and 3B), indicating that the efficacy of DCV was not due to a toxic response.

A previous study showed that MSU increased IL-1*β* levels and caused massive neutrophil influx in an NLRP3 inflammasome-dependent manner [41-42]. Thus, the effect of DCV on MSU-induced peritonitis was examined. The results presented that DCV efficiently suppressed MSU-induced IL-1*β* production and caspase-1 cleavage in *Nlrp3*$^{+/+}$ mice rather than *Nlrp3*$^{-/-}$ mice (Figs. 5G−5I). Collectively, DCV ameliorated inflammation in a mouse model through inhibiting the NLRP3 inflammasome *in vivo*.

Discussion

In the current study, we demonstrates that DCV acts as a potent and selective NLRP3 inflammasome inhibitor. More importantly, DCV exhibits inhibitory effect against NLRP3 inflammasome activation in mice *in vivo* (Fig. 6). DCV can serve as a versatile therapeutic agent against NLRP3-driven diseases.

DCV has shown antioxidant and anti-inflammatory activity in several experimental disease models [27]. Although previous studies indicated that DCV suppressed the NF-*κ*B and MAPK signaling pathways and reduced the production of

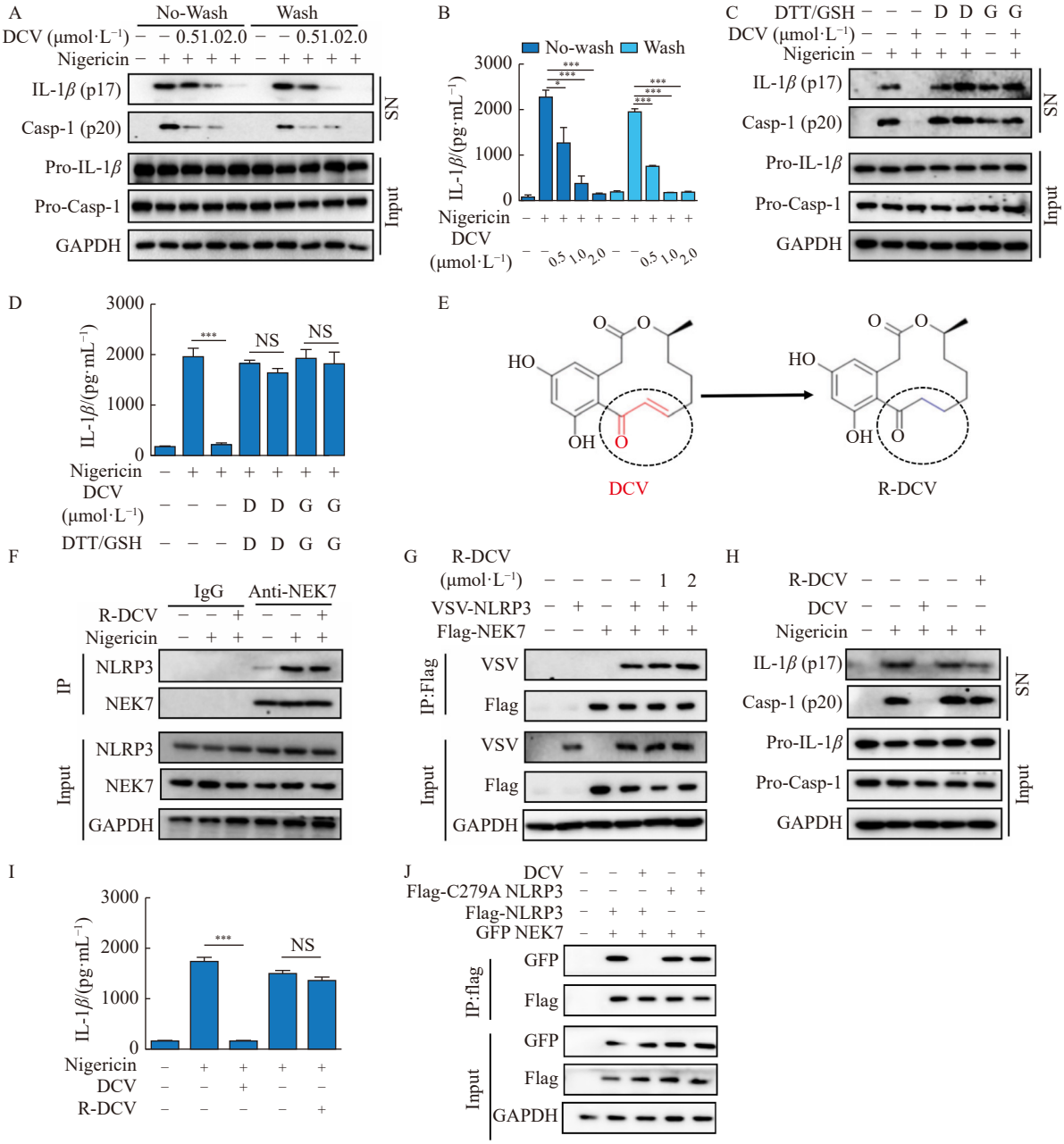

Fig. 4 DCV suppresses NLRP3 inflammasome activation in a Michael acceptor-dependent manner. (A) LPS-primed BMDMs were treated with DCV (0.5, 1.0 or 2.0 μmol·L^{-1}) for 15 min, washed three times, and then stimulated with nigericin. Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (B) Production of IL-1*β* in SN from the LPS-primed BMDMs described in (A). (C) LPS-primed BMDMs were preincubated with 0.05 mmol·L^{-1} DTT, 0.2 mmol·L^{-1} GSH and DCV and then stimulated with nigericin. Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (D) Production of IL-1*β* in SN from the LPS-primed BMDMs described in (C). (E) The structure of DCV without a C=C double bond (R-DCV). (F) LPS-primed BMDMs were treated with R-DCV (2.0 μmol·L^{-1}) and then stimulated with nigericin, and the endogenous NLRP3-NEK7 interaction was analyzed. (G) Co-IP analysis of the interaction of VSV-NLRP3 and Flag-NEK7 in HEK-293T cells pretreated with R-DCV (2.0 μmol·L^{-1}). (H) LPS-primed BMDMs were treated with DCV or RDCV (2.0 μmol·L^{-1}) and then stimulated with nigericin. Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) in SN and pro-IL-1*β* and pro-caspase-1 (p45) in Input. (I) Production of IL-1*β* in SN from the LPS-primed BMDMs described in (H). (J) Co-IP analysis of the interaction between NEK7 and WT or C279A mutant NLRP3 in HEK-293T cells. Data are expressed as the means ± SD of at least three independent experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ *vs* control

inflammatory cytokines, such as TNF-*α* and IL-6 [32-33], the molecular mechanism of DCV is not clear. The *in vitro* and *in vivo* results revealed that DCV acted as a potent NLRP3 inflammasome inhibitor and exhibited beneficial therapeutic effect on LPS-induced systemic inflammation and MSU-induced peritonitis. However, the beneficial therapeut-

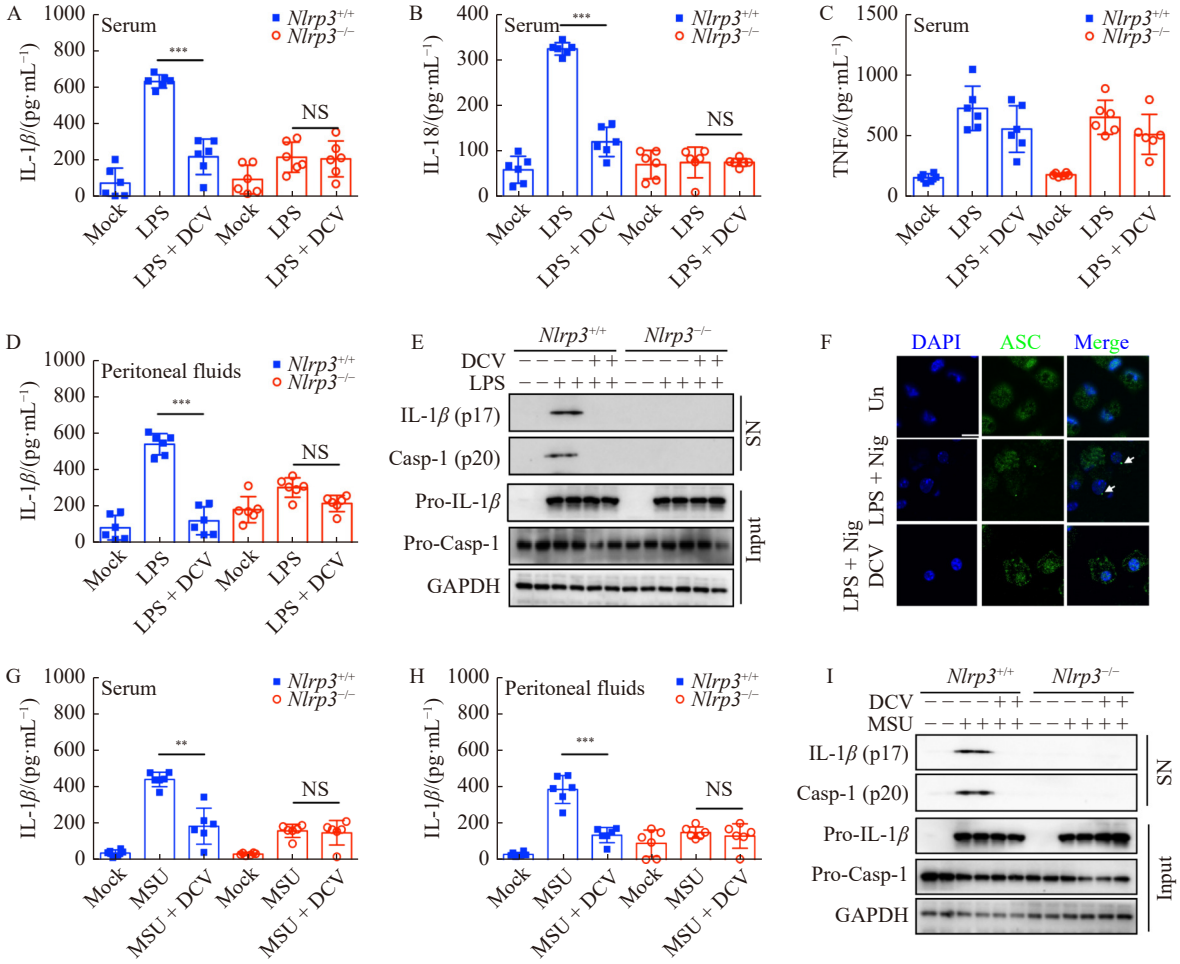

Fig. 5 DCV suppresses NLRP3-dependent inflammation *in vivo* (A−C) *Nlrp3*$^{+/+}$ and *Nlrp3*$^{-/-}$ mice were intraperitoneally injected with LPS with and without DCV. Serum IL-1*β* (A), IL-18 (B), and TNF-*α* (C) were measured. (D) Production of IL-1*β* in the peritoneal fluid of the LPS-treated mice described in (A). (E) Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) and pro-IL-1*β* and pro-caspase-1 (p45) in *Nlrp3*$^{+/+}$ and *Nlrp3*$^{-/-}$ mice administered with LPS in the presence of DCV. (F) Representative immunofluorescence images of ASC speck formation in peritoneal macrophages stimulated with nigericin in the presence of DCV. (G−H) ELISA of IL-1*β* in the serum (E) or peritoneal fluid (F) of *Nlrp3*$^{+/+}$ or *Nlrp3*$^{-/-}$ mice intraperitoneally injected with MSU with and without DCV. (I) Western blot analysis of IL-1*β* (p17) and caspase-1 (p20) and pro-IL-1*β* and pro-caspase-1 (p45) in *Nlrp3*$^{+/+}$ and *Nlrp3*$^{-/-}$ mice administered with MSU crystals in the presence of DCV. Data are expressed as the means ± SD of at least three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control

ic effect of DCV was absent in *Nlrp3*$^{-/-}$ mice, suggesting that the anti-inflammatory effect of DCV depends on the NLRP3 inflammasome *in vivo*. Therefore, DCV may be a potential NLRP3 inhibitor for the treatment of NLRP3-driven inflammatory diseases.

Increasing evidence has shown that several compounds block NLRP3 inflammasome activation in cells and animal models; however, nonspecific effects have limited its application [43-47]. Previous studies indicated that several compounds suppressed NLRP3 activation by inhibiting oxidative stress. However, our data revealed that DCV did not affect oxidative stress. The protein complex containing NLRP3, ASC, and caspase-1 plays an essential role in NLRP3 inflammasome activation [40]. The interaction between NLRP3 and NEK7 is a key step for NLRP3 inflammasome assembly [48]. Our results demonstrated that DCV impaired the NLRP3-NEK7 interaction. NEK7, a new inflammasome component, binds to the leucine-rich repeat domain of the NLRP3 protein and is required for NLRP3 inflammasome assembly rather than NLRC4 or AIM2 protein assembly [48, 49]. These results suggest that NEK7 is necessary for activation of the NLRP3 inflammasome. Our findings are consistent with these results showing that DCV selectively suppresses NLRP3 inflammasome activation but cannot inhibit NLRC4 or AIM2 inflammasome activation. Collectively, our data indicate that DCV suppresses the activation of the NLRP3 inflammasome by interrupting the NLRP3-NKE7 interaction, suggesting that the NLRP3-NKE7 interaction is a potential target for treatment.

An interesting question is how DCV inhibits the NLRP3-NKE7 interaction. Several lines of evidence have implied that the *α*,*β*-unsaturated carbonyl unit of DCV is required for NLRP3 inflammasome inhibition. First, DCV still inhibits IL-

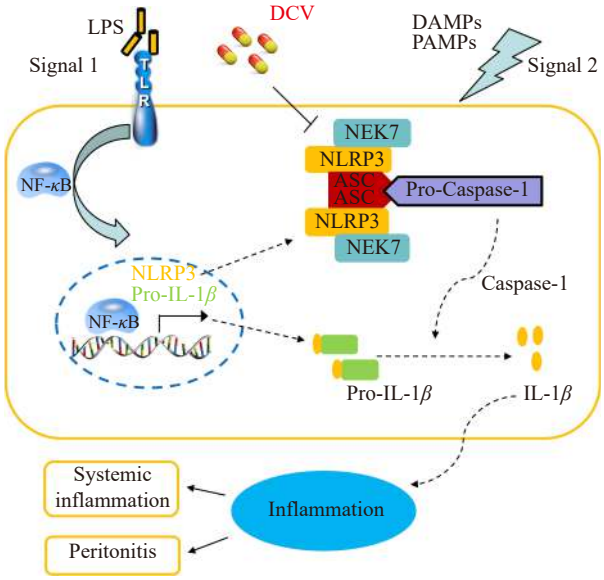

Fig. 6 Schematic representation of the mechanism of action of DCV

1β production and caspase-1 activation after washout, implying that the inhibitory role of DCV is irreversible. Second, either DTT or GSH effectively ameliorates NLRP3 inflammasome inactivation induced by DCV. Third, R-DCV, the reduced form of DCV, does not affect the NEK7-NLRP3 interaction or subsequent NLRP3 inflammasome activation. Cysteine 279 of NLRP3 in NACHT domain is essential for NLRP3 inflammasome activation [21], and our results indicate that DCV does not suppress the interaction between the C279A NLRP3 mutant and NKE7. The detailed mechanism of how DCV binds to NLRP3 must to be investigated in future studies by biochemical and structural approaches.

Taken together, our data demonstrate that DCV can inhibit NLRP3 inflammasome activation, thus attenuating inflammatory response. Importantly, DCV attenuates NLRP3 inflammasome-related human diseases in the mouse model *in vivo*. As NLRP3 inflammasome activation is involved in several inflammatory diseases, DCV may be a suitable drug candidate for the treatment of NLRP3-driven diseases.

Supporting Information

Supporting information of this paper can be requested by sending E-mail to the corresponding author.

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