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Lupeol inhibits LPS-induced NF-kappa B signaling in intestinal epithelial cells and macrophages, and attenuates acute and chronic murine colitis

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ABSTRACT

Aims: Lupeol, a natural pentacyclic triterpene, exhibits anti-inflammatory effects. However, its role in colitis has not been investigated. In the present study, we evaluated the effect of lupeol on the NF-κB signaling pathway and experimental colitis in mice.
Main methods: The human intestinal epithelial cells (IECs) COLO 205 and the murine macrophages RAW 264.7 were pretreated with lupeol and then stimulated with lipopolysaccharide (LPS). The production of inflammatory cytokines (IL-8 from COLO 205; IL-6, IL-12 and TNF-α from RAW 264.7) was determined by ELISA. The effect of lupeol on NF-κB pathway was examined by Western blot analysis of IκBα phosphorylation/degradation and an electrophoretic mobility shift assay (EMSA). For in vivo studies, dextran sulfate sodium (DSS)-induced acute colitis model and chronic colitis model in IL-$10^{-/-}$ mice were used. Colitis was quantified by disease activity index, colon length and histologic evaluation.
Key findings: Lupeol strongly suppressed pro-inflammatory cytokine production in IECs and murine macrophages. It also inhibited LPS-induced IκBα phosphorylation/degradation and the DNA binding activity of NF-κB. The oral administration of lupeol significantly reduced the colitis activity and histologic scores in both acute and chronic murine colitis models. Furthermore, the up-regulation of IκBα phosphorylation in the colonic mucosa was attenuated in lupeol-treated mice.
Significance: Lupeol blocks the NF-κB signaling in IECs and murine macrophages, and attenuate experimental murine colitis. These findings suggest that lupeol is a potential therapeutic agent for inflammatory bowel disease.

1. Introduction

The pathophysiology of inflammatory bowel disease (IBD) is still unclear, but an unregulated immune response of intestinal mucosa to commensal bacterial component, such as lipopolysaccharide (LPS), could be one of the main pathogenesis of IBD [20]. Because the gut-microbial homeostasis mainly depends on the immune tolerance of tissue resident macrophage and the barrier function of intestinal epithelial cells (IECs) [31], the bacterial translocation across the intestinal barrier and the subsequent activation of macrophage by LPS has been known as the critical pathway in unregulated immune response of intestinal mucosa [23]. The main key regulator of immune response of intestinal mucosa has been regarded as the nuclear transcription factor-κB (NF-κB) [1], since NF-κB activation was founded in mucosal macrophage and colonic epithelial cells of IBD patients [22]. Furthermore, the agents inhibiting NF-κB signal pathway had suggested as a new therapeutic agent to IBD [12].

Recently, there has been growing interest for identifying an alternative therapeutic agent to IBD from the natural food substances or herbal medicine, reflecting the higher compliance of patients and the general belief in the safety of natural substance [9]. Lupeol ($C_{30}H_{50}O$), a natural pentacyclic triterpene, is found in vegetables, edible fruits, and medical plants such as mango, Japanese pear, olive, cabbage, green peppers, *Aloe vera*, and ginseng oil [18,25,28]. Several studies have reported that lupeol possesses strong anti-inflammatory and anti-cancer activities [15,25,30]. However, the effect of lupeol on colonic inflammation has not been investigated, and the underlying mechanism of lupeol on NF-κB signal pathway in IECs and macrophages also has not been clearly provided.

To explore the anti-inflammatory mechanism of lupeol, we evaluate the effect on IκBα phosphorylation and DNA binding activity of the NF-κB in IECs and macrophages, and then assess the anti-inflammatory effect of lupeol in in vivo by treating experimental murine colitis with oral administration of lupeol. In this study, two murine model of colitis were used complimentarily: 1) a prevention of acute colitis of dextran sulfate

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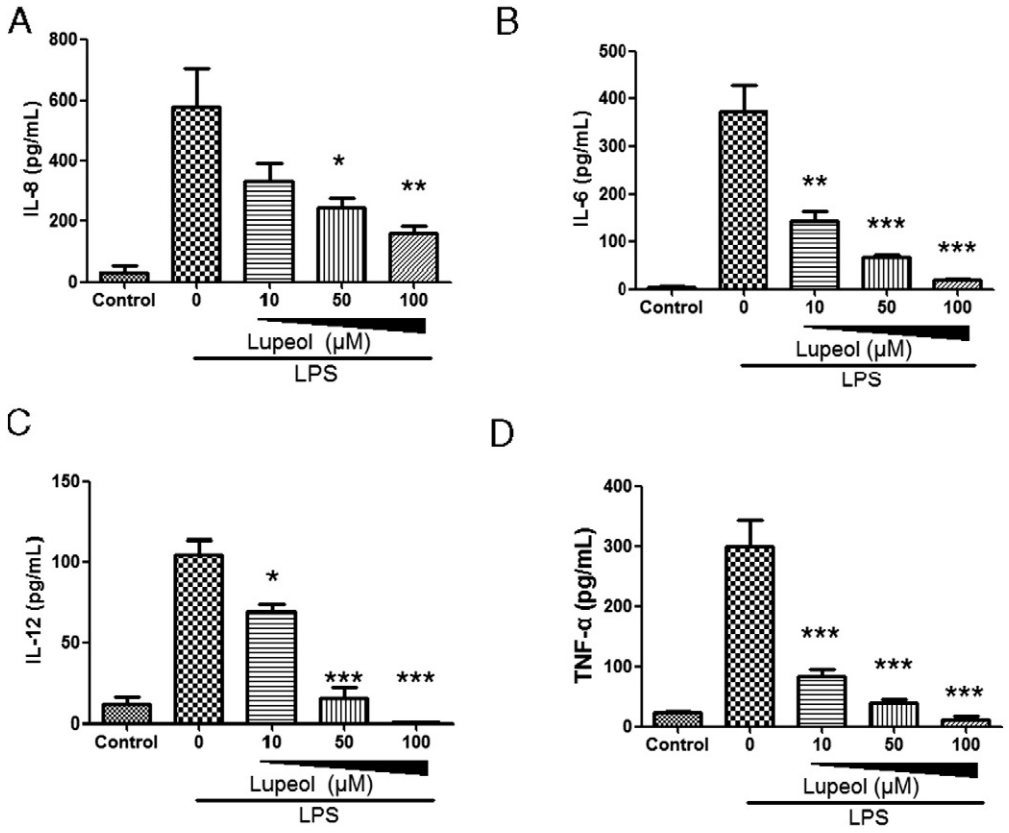

Fig. 1. Lupeol suppressed pro-inflammatory cytokine production. COLO 205 cells and RAW 264.7 cells were pretreated with three doses (i.e., 10 or 50 or 100 μM/ml) of lupeol for 24 h, followed by stimulation with 10 μg/ml lipopolysaccharide (LPS) for 4 h. (A) The secretion of IL-8 from COLO 205 cells was measured using ELISA. (B, C, D) The secretion of IL-6, IL-12 and TNF-α from RAW 264.7 cells was measured using ELISA. Lupeol strongly suppressed pro-inflammatory cytokine production in both cells. The data are representative of more than three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with LPS alone.

sodium (DSS); 2) a treatment of chronic colitis in IL-10-deficient (IL-$10^{-/-}$) mice.

2. Materials and methods

2.1. Ethical considerations

All experiments using mice were approved by the Institutional Animal Care and Use Committee (IACUC) at the Seoul National University Hospital (No. 14-0229) and the Seoul National University (No. SNU-140211-2).

2.2. Mice

Specific pathogen free (SPF) C57BL/6 Wide type (WT) mice were obtained from Orient (Seongnam, Korea) and SPF IL-$10^{-/-}$ C57BL/6 mice were provided by the Biomedical Center for Animal Resource Development (BCARD) of Seoul National University (Seoul, Korea) [4,16]. The

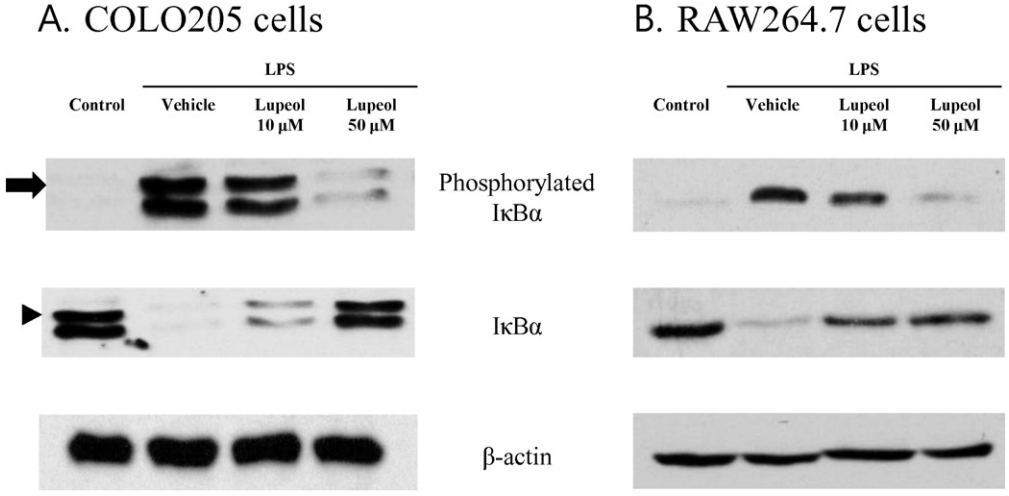

Fig. 2. Lupeol blocked LPS-induced IκBα phosphorylation and degradation. (A, B) Western blot analysis of IκBα (black triangle) and phosphorylated IκBα (black arrow) in COLO 205 cells (A) and RAW 264.7 cells. Lupeol pretreatment attenuated LPS-induced IκBα phosphorylation and degradation. The data are representative of more than three independent experiments.