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A new mechanism of inhibition of IL-1β secretion by celastrol through the NLRP3 inflammasome pathway

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ABSTRACT

The NLRP3 (NOD-like receptor protein 3) inflammasome is a caspase-1-containing multiprotein complex that controls the release of IL-1β and has been associated with the development of inflammatory diseases. Celastrol, a pharmacologically active ingredient extracted from *Tripterygium wilfordii Hook*, has anti-inflammatory activities based on its inhibition of IL-1β secretion. The purpose of the present study was to investigate the possible modulation of NLRP3 inflammasome-mediated IL-1β and IL-18 release from macrophages by celastrol. It was shown that celastrol significantly reduced the secretion of IL-1β and IL-18 by inhibiting the expression of NLRP3 and the cleavage of caspase-1 in lipopolysaccharide (LPS)/ATP-induced macrophages. In addition, celastrol suppressed pyroptosis in macrophages, demonstrated by caspase-1 activation, LDH leakage and PI uptake assays. Furthermore, these inhibitory effects of celastrol were found to be at least partially achieved by decreasing the up-regulation of reactive oxygen species generation and NF-κB activation. Taken together, these findings suggested a new anti-inflammation mechanism of celastrol through inhibition of the NLRP3 inflammasome.

1. Introduction

It is commonly accepted that pro-inflammatory cytokines released from macrophages are key mediators of the host immune response and play critical roles in numerous inflammatory pathologies. The IL-1 family has been recognized to play important roles in inflammation, and it plays a significant role in innate immune defense (van de Veerdonk and Netea, 2013). There are several cytokines belonging to the IL-1 family, such as IL-1α, IL-1β, IL-18 and IL-33. In particular, IL-1β is the most characterized cytokine among the IL-1 family, and its effects have been extensively studied (Dinarello, 2011; Qi et al., 2014).

In macrophages, IL-1β has a unique molecular mechanism for its maturation by an intracellular multiprotein complex called inflammasome. Pro-IL-1β is processed by the NLRP3 inflammasome; then, the mature IL-1β is secreted. The NLRP3 inflammasome consists of caspase-1, responsible for the cleavage of pro-IL-1β, a member of the NLR family, which acts as the sensor component of the inflammasome and ASC, a CARD/PYD protein that serves as a docking and activation platform for caspase-1 (Cai et al., 2016; Martinon et al., 2002).

The molecular mechanisms for the assembly and activation of the NLRP3 inflammasome have been explored over the years. Previous studies have indicated that this process requires dual signals. One is the priming signal that stimulates TLR4 and then enhances the NF-κB-driven transcription of NLRP3 (Davis et al., 2011; Schroder and Tschopp, 2010). The second signal promotes NLRP3 to form a protein complex with ASC containing a caspase recruitment domain and then induces the activation of caspase-1 (Dowling and O'Neill, 2012). It is also well-recognized that the generation of reactive oxygen species, potassium (K^+) efflux induced by extracellular ATP, and the release of cathepsin B can activate the NLRP3 inflammasome (Jin and Flavell, 2010).

A strong link between the NLRP3 inflammasome and inflammatory diseases, such as arthritis, cancer, type 2 diabetes and liver diseases, is becoming increasingly evident (Abderrazak et al., 2016; Choulaki et al., 2015; Lebeaupin et al., 2015; Muzes and Sipos, 2015). Thus, identification of endogenous mechanisms that control the deactivation of the NLRP3 inflammasome or novel therapeutic agents that inhibit NLRP3 activation could be a strategy in the treatment of inflammation-related diseases.

Celastrol (Fig. 1A), a triterpene, is a pharmacologically active ingredient extracted from *Tripterygium wilfordii Hook*, a traditional Chinese medicinal plant known as the “Thunder of God Vine”. Celastrol

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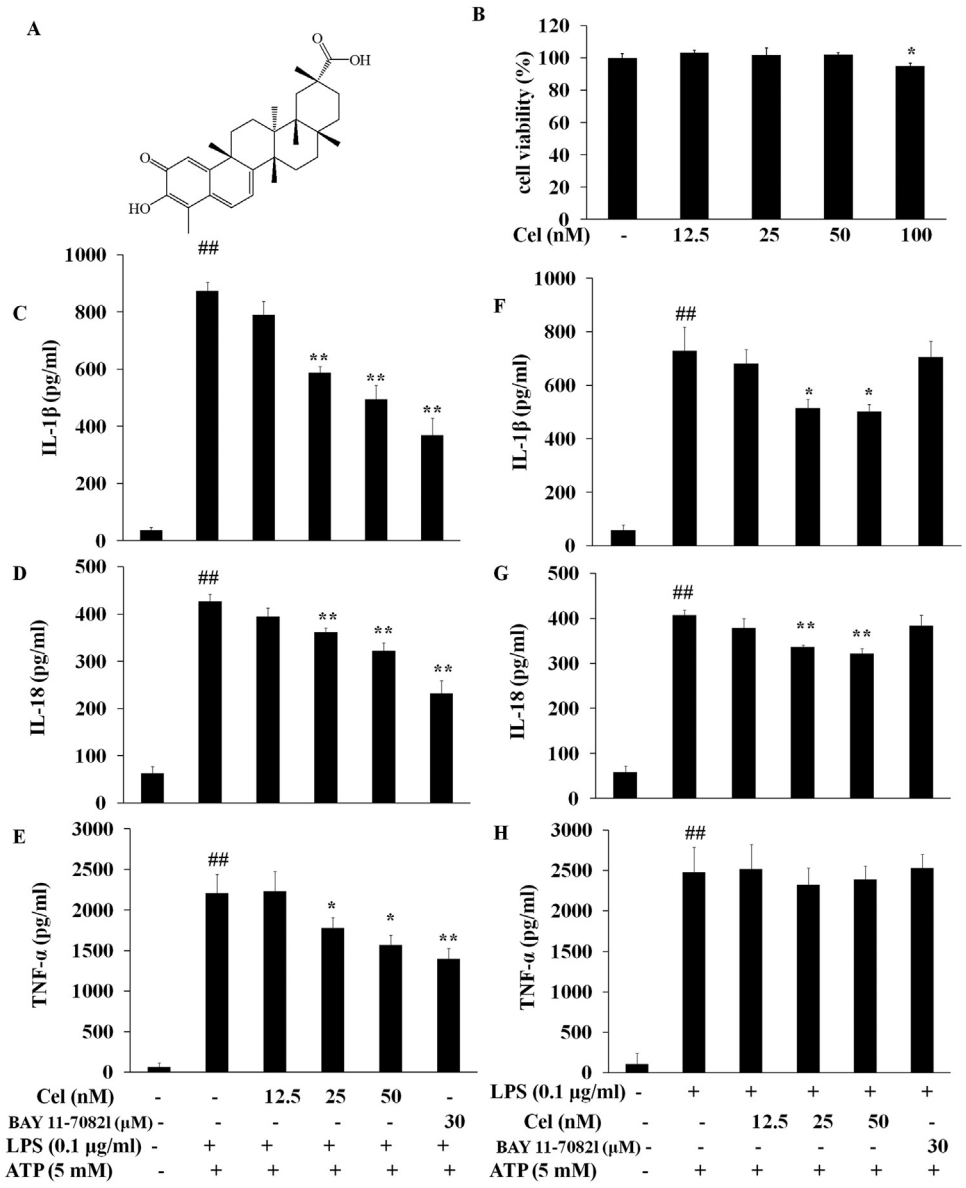

Fig. 1. Celastrol suppresses IL-1β and IL-18 secretion in J774A.1 macrophages. (A) The structure of celastrol. (B) Cell viability was determined with the MTT assay at the indicated concentrations of celastrol. [(C) IL-1β, (D) IL-18 and (E) TNF-α concentrations in culture supernatant] J774A.1 macrophages were pretreated with celastrol (12.5, 25 or 50 nM) or BAY 11-7082 for 1 h and then cultured with 0.1 μg/ml LPS for 12 h, followed by 30 min of incubation with 5 mM ATP. [(F) IL-1β, (G) IL-18 and (H) TNF-α concentrations in culture supernatant] J774A.1 macrophages were stimulated with LPS (0.1 μg/ml) for 12 h, then treated with celastrol (12.5, 25 or 50 nM) or BAY 11-7082 for 1 h, followed by 30 min of incubation with 5 mM ATP. The concentrations of IL-1β, IL-18 and TNF-α in the culture supernatant were analyzed by ELISA. The data represent the means ± S.D. of triplicate tests. $^{\#\#}P < 0.01$ compared with the control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the LPS and ATP groups.

exerts powerful effects on chronic inflammation, autoimmune diseases, cancer and obesity (Astry et al., 2015; Li et al., 2013; Liu et al., 2015, 2016; Sanna et al., 2015). An increasing amount of evidences indicate that celastrol exerts anti-inflammatory properties because of its inhibition of IL-1β secretion (Cascao et al., 2012; Kim et al., 2009a). However, the detailed mechanisms are still poorly understood. Since secretion of IL-1β is tightly regulated by the NLRP3 inflammasome, we hypothesized that celastrol might regulate NLRP3 inflammasome activation. In this study, we demonstrate that celastrol inhibits NLRP3 inflammasome activation by reducing both the priming and activating signals of the NLRP3 inflammasome in macrophages.

2. Materials and methods

2.1. Materials

The mouse macrophage cell line J774A.1 was purchased from the American Type Culture Collection (Rockville, MD, USA). Celastrol (Fig. 1) was purchased from KMST Pharmaceutical Technology Development Co., Ltd. (Tianjing, PR China). Its molecular formula is $C_{29}H_{38}O_4$, and its molecular weight is 450.61 Da. LPS (Escherichia coli O55:B5) and 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) were purchased from Sigma (St. Louis, MO, USA). RPMI 1640 and fetal bovine serum were obtained from Gibco (Gibco-BRL, Gaithersburg, MD, USA). The enzyme-linked immunosorbent assay (ELISA) kits for mouse TNF-α, IL-1β and IL-18 were purchased from Neobioscience (Beijing, China). Reactive oxygen species scavenger NAC, NF-κB inhibitor BAY 11–7082, caspase inhibitor Z-VAD-FMK, the LDH cytotoxicity assay detection kit and Caspase-1 activity kit were purchased from Beyotime (Beijing, China). An antibody against caspase-1 was obtained from Abcam (Cambridge, UK). Antibodies against NLRP3, NEK-7, ASC, caspase-1, IL-1β, phospho-NF-κB p65, NF-κB p65, phospho-IκBα, IκBα and GAPDH were purchased from Cell Signaling Technology (Beverly, MA, USA). Other reagents were of commercially available analytical grade.

2.2. Cell culture and treatment

The cells were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, streptomycin (100 U/ml) and penicillin (100 U/ml). The cells were kept at 37 °C in a 5% CO_2 incubator. For the measurement of the priming signal that stimulates the activation of the NLRP3 inflammasome, cells (1×10^5/ml) were seeded in 96-well