International Immunopharmacology 57 (2018) 172–180

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Andrographolide suppresses TRIF-dependent signaling of toll-like receptors by targeting TBK1

Ah-Yeon Kim, Hyun-Jin Shim, Hyeon-Myeong Shin, Yoo Jung Lee, Hyeonjeong Nam, Su Yeon Kim, Hyung-Sun Youn*

Department of Biomedical Laboratory Science, College of Medical Sciences, SoonChunHyang University, Chungnam, Asan 31538, Republic of Korea

ARTICLE INFO

Keywords:
Toll-like receptors
Andrographolide
Inflammation
TRIF
TBK1

ABSTRACT

Toll-like receptors (TLRs) play a crucial role in danger recognition and induction of innate immune response against bacterial and viral infections. The TLR adaptor molecule, toll-interleukin-1 receptor domain-containing adapter inducing interferon-β (TRIF), facilitates TLR3 and TLR4 signaling, leading to the activation of the transcription factor, NF-κB and interferon regulatory factor 3 (IRF3). Andrographolide, the active component of *Andrographis paniculata*, exerts anti-inflammatory effects; however, the principal molecular mechanisms remain unclear. The objective of this study was to investigate the role of andrographolide in TLR signaling pathways. Andrographolide suppressed NF-κB activation as well as COX-2 expression induced by TLR3 or TLR4 agonists. Andrographolide also suppressed the activation of IRF3 and the expression of interferon inducible protein-10 (IP-10) induced by TLR3 or TLR4 agonists. Andrographolide attenuated ligand-independent activation of IRF3 following overexpression of TRIF, TBK1, or IRF3. Furthermore, andrographolide inhibited TBK1 kinase activity *in vitro*. These results indicate that andrographolide modulates the TRIF-dependent pathway of TLRs by targeting TBK1 and represents a potential new anti-inflammatory candidate.

1. Introduction

Inflammation is a complex set of reactions that occurs following microbial infection or tissue damage, which mainly result in redness, swelling, fever, and pain of organs [1]. Chronic inflammation is a critical step in the cascade of events leading to the development of several inflammatory diseases. Toll-like receptors (TLRs) are pathogen recognition receptors that play a key role in the nonspecific or innate immune defense, particularly in inflammatory response against invading microbial pathogens, including bacteria, virus, fungi, and parasites [2,3]. Recent studies suggest the role of TLRs in various chronic inflammatory diseases [4].

Currently, 13 groups of TLRs in mammalian cells have been identified: 10 in humans, 13 in mice, and > 20 in non-mammalian genomes [2]. TLRs recognize a wide variety of pathogen-associated molecular patterns (PAMPs). The TLR1/2/4/5/6 receptors recognize microbial patterns that are accessible on the cell surface. Endosomal TLR3/7/9 receptors detect microbial nucleic acids. TLRs are an important link between the innate and adaptive immunity. TLR signaling pathways involve four different toll-interleukin 1 receptor (TIR)-domain-containing adaptor proteins (TIRAP) such as myeloid differentiation primary response 88 (MyD88), TIRAP/MyD88 adaptor-like (MAL), TIR domain-containing adapter inducing interferon-β (TRIF), and TRIF-related adaptor molecule [3]. Whereas MyD88 is used by TLR2, TLR5, TLR7, TLR8, and TLR9, TRIF is used by TLR3. TLR4 uses both MyD88 and TRIF-dependent pathways for the induction of proinflammatory cytokines and IFN-stimulated genes [5]. TLR signaling pathways are collectively responsible for the activation of IKKs, which in turn, activate a suite of transcription factors, such as NF-κB and members of the IRF family [6,7].

Recent studies have investigated the possible role of natural compounds to effectively prevent inflammatory diseases [8–10]. *Andrographis paniculata* [(Burn.f) Nees] has been widely used for long periods as traditional medicine in China, India, and Thailand [11]. Andrographolide (Fig. 1) is the active component of *Andrographis paniculata*, a medicinal plant from the Acanthaceae family. Pharmacological studies have shown that andrographolide is cost-effective and exhibits antiviral, anti-bacterial, anti-cancer, and anti-inflammatory properties with low toxicity [12–15]. Andrographolide inhibits NF-κB activation, inducible nitric oxide synthase (iNOS) expression, and prostaglandin E2 production induced by lipopolysaccharide (LPS) [16,17]. However, the potential pharmacological mechanisms of andrographolide remain unclear.

Dysregulated activation of TLRs is known to be closely linked to the

* Corresponding author at: Department of Biomedical Laboratory Science, College of Medical Sciences, Soonchunhyang University, Asan-Si, Chungnam 336-745, Republic of Korea.
E-mail address: hyoun@sch.ac.kr (H.-S. Youn).

https://doi.org/10.1016/j.intimp.2018.02.019
Received 15 January 2018; Received in revised form 15 February 2018; Accepted 22 February 2018

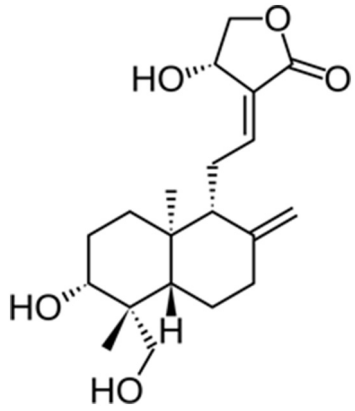


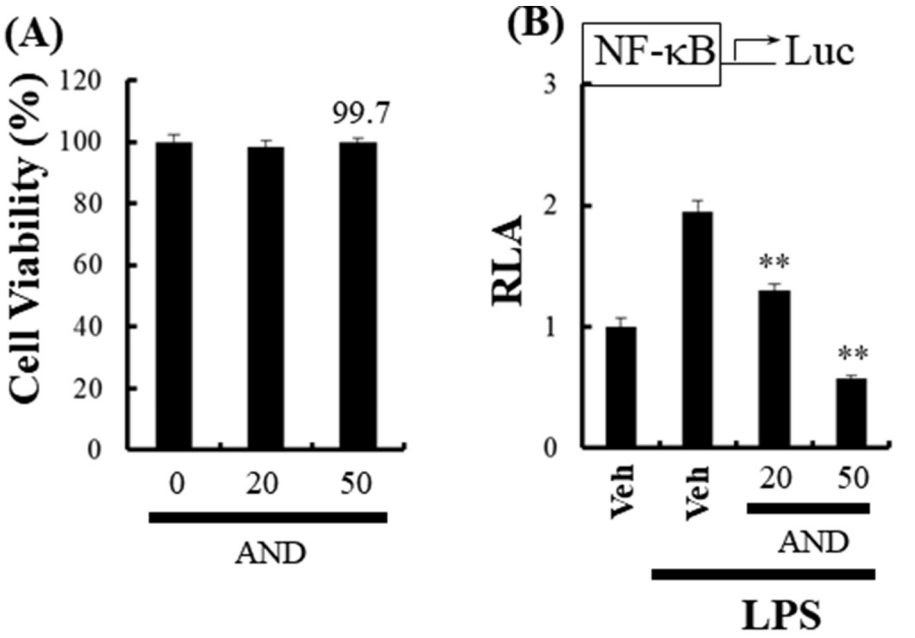
Fig. 1. The structure of andrographolide.

development of chronic inflammatory diseases. Therefore, TLRs signaling pathways represent potential therapeutic targets for many chronic inflammatory diseases. While andrographolide affects various cellular processes, data currently fail to fully explain its anti-inflammatory mechanism. The aim of this study was to elucidate the molecular targets of andrographolide in TLR signaling pathways.

2. Materials and methods

2.1. Reagents

Andrographolide was purchased from Sigma-Aldrich (St. Louis, MO). LPS was purchased from List Biological Lab (Campbell, CA). Macrophage-activating lipopeptide of 2 kDa (MALP-2) was purchased from Alexis Biochemical (San Diego, CA). Poly[I:C] was obtained from Amersham Biosciences (Piscataway, NJ). COX-2 and β-actin antibodies



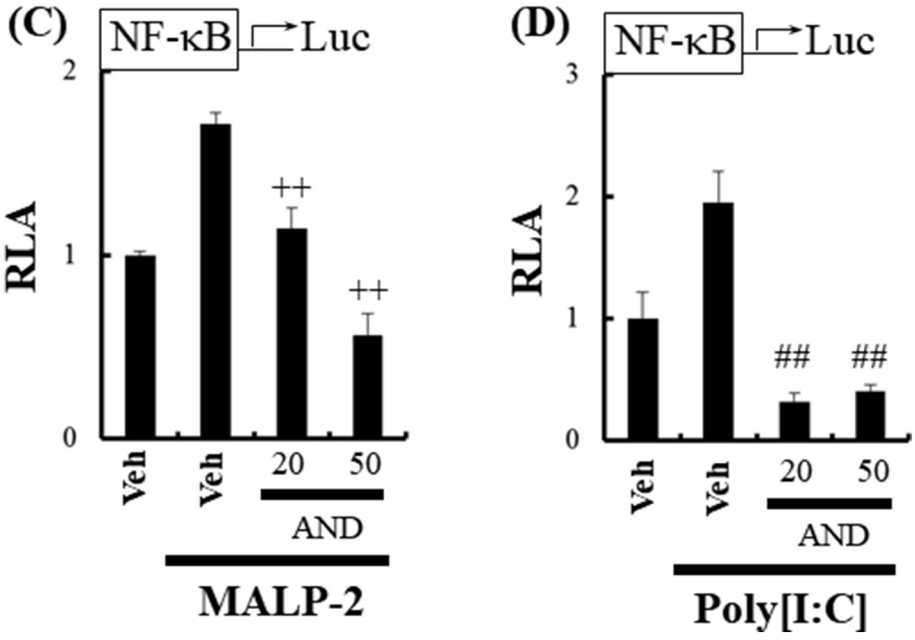


Fig. 2. Andrographolide inhibits NF-κB activation. A) RAW264.7 cells were treated with andrographolide (20, 50 μM) for 4 h. Twenty microliters of the CellTiter 96 AQ_{ueous} One Solution Reagent was added directly to culture wells. The plate was incubated at 37 °C for 4 h in a humidified, 5% CO_2 atmosphere. The absorbance was recorded at 490 nm with a 96-well plate reader. *B–D*) RAW264.7 cells were transfected with NF-κB luciferase reporter plasmid, and were pre-treated with andrographolide (20, 50 μM) for 1 h, and then treated with LPS (10 ng/ml) (B), MALP-2 (10 ng/ml) (C), or Poly[I:C] (10 μg/ml) (D) for an additional 8 h. Cell lysates were prepared and luciferase and β-galactosidase enzyme activities were measured as described in Materials and methods. Relative luciferase activity (RLA) was normalized with β-galactosidase activity. Values are mean ± SEM ($n = 3$). *, denotes a result that is significantly different from LPS alone, $p < 0.01$ (**) (B). +, denotes a result that is significantly different from MALP-2 alone, $p < 0.01$ (++) (C). #, denotes a result that is significantly different from Poly[I:C] alone, $p < 0.01$ (##) (D). Veh, vehicle; AND, andrographolide.