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Anti-inflammatory effect of desoxo-narchinol-A isolated from *Nardostachys jatamansi* against lipopolysaccharide

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ABSTRACT

We previously reported that *Nardostachys jatamansi* (NJ) exhibits anti-inflammatory activity against lipopolysaccharide (LPS). However, the active compound in NJ is unknown. Therefore, here, we examined the effects of desoxo-narchinol-A (DN) isolated from NJ against LPS-induced inflammation. To demonstrate the anti-inflammatory effect of DN against LPS, we used two models; murine endotoxin shock model for in vivo model, and peritoneal macrophage responses for in vitro. In endotoxin shock model, DN was administrated intraperitoneally 1 h before LPS challenge, then we evaluated mice survival rates and organ damages. Pretreatment with DN (0.05 mg/kg, 0.1 mg/kg, or 0.5 mg/kg) dramatically reduced mortality in a murine LPS-induced endotoxin shock model. Furthermore, DN inhibited tissue injury and production of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α), in the liver and lung. In in vitro macrophage model, we examined the inflammatory mediators and regulatory mechanisms such as mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NF-κB). DN inhibited the production of inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and its derivative nitric oxide (NO), cyclooxygenase-2 (COX-2), prostaglandin E_2 (PGE_2), IL-1β, IL-6 and TNF-α and H3 protein acetylation in murine peritoneal macrophages. DN also inhibited p38 activation, but not extracellular signal-regulated kinase (ERK), c-jun NH_2-terminal kinase (JNK), and NF-κB. These results suggest that DN from NJ exhibits protective effects against LPS-induced endotoxin shock and inflammation through p38 deactivation.

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1. Introduction

Inflammation is a complex pathophysiological process that involves activation of various immune cells such as macrophages [1–3]. During the inflammatory process, pro-inflammatory cells (mainly activated macrophages) are responsible for most of the cellular and molecular pathophysiology of sepsis because they produce cytokines and other pro-inflammatory molecules, including interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), prostaglandin E_2 (PGE_2), and nitric oxide (NO) [4–6]. Among a variety of inflammatory mediators, two of the most prominent are NO and PGE_2, which are the products of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively [7].

Nardostachys jatamansi (NJ) belonging to the Valerianaceae family is a small, endangered, perennial herb. NJ is originated throughout the

Abbreviations: COX-2, cyclooxygenase-2; DN, desoxo-narchinol-A; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; EtOAc, ethyl acetate; FBS, fetal bovine serum; H&E, hematoxylin and eosin; HPRT, hypoxanthine guanine phosphoribosyl-transferase; Iκ-Bα, inhibitory kappa-Bα; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun NH2-terminal kinase; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MPLC, medium pressure liquid chromatography; mRNA, messenger RNA; MTT, tetrazolium compound 3-(4,5dimethylthiazol)-2,5-diphenyl tetrazolium bromide; NF-κB, nuclear factor kappa B; NJ, nardostachys jatamansi; NO, nitric oxide; PBST, phosphate buffered saline with Tween 20; PCR, polymerase chain reaction; PGE_2, prostaglandin E_2; RPMI-1640, Roswell Park Memorial Institute culture medium; SB, sb203580; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TG, thioglycollate; TLC, thin layer chromatography; TLR, Toll-like receptor; TMB, tetramethylbenzidine; TNF-α, tumor necrosis factor alpha.

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Himalayas and is widely used in several Asian countries to treat palpitations, mental disorders, insomnia, epilepsy, blood disorders, disorders of the circulatory system and Herpes infection [8,9]. Various sesquiterpenes, such as jatamansic acid, jatamansone, lignans, and neolignans, are present in the roots of these plants [10,11]. We previously reported that NJ effectively protects against inflammation, especially lipopolysaccharide (LPS)-induced inflammation and endotoxin shock such as sepsis [12,13]. However, the NJ-derived compounds that exhibit anti-inflammatory activity against LPS have not been investigated. Therefore, the aim of this study was to investigate the anti-inflammatory effect of desoxo-narchinol A (DN) isolated from NJ in murine peritoneal macrophages and in a mouse endotoxin shock model with LPS. Accordingly, we performed in vivo and in vitro analysis to investigate whether DN has beneficial effects. Furthermore, to investigate the mechanisms regulating DN, we examined the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NF-κB).

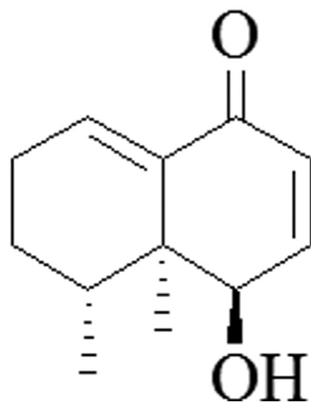
2. Materials and methods

2.1. Extraction and isolation

NJ was extracted using hot H_2O. NJ extract (15 g) was dissolved in H_2O and partitioned with ethyl acetate (EtOAc) followed by n-butanol. The EtOAc-soluble fraction (2.2 g) was separated on a silica gel column (6.5 × 60 cm, 70–230 mesh, Merck) using n-hexane/EtOAc (4:1–1:2) as the eluent to obtain six fractions (Fr. 1–6). Fraction 2 (468.7 mg) was separated on a reverse-phase (S-75 μm, YMC Gel ODS-A, YMC Co. Ltd., Japan) column (3 × 25 cm) with MeOH/H_2O (1:1) to yield an additional four fractions (Fr. 2–1–2–4). Fraction 2–2 (137.7 mg) was purified using medium pressure liquid chromatography (MPLC) (column: SI-40 A, 11 mm × 300 mm) with dichloromethane/MeOH (30:1) as the eluent to produce DN (43.7 mg) (Scheme 1A). The spectral data were identical to those reported in the literature [14].

2.2. Chemicals and reagents

Roswell Park Memorial Institute culture medium (RPMI-1640), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). Enzyme-linked immunosorbent assay (ELISA) kits for detection of mouse IL-1β, IL-6, and TNF-α were purchased from R&D Systems (Minneapolis, MN, USA). LPS from *Escherichia coli* 055:B5 was purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Antibodies against total and phospho-specific MAPKs (p38; extracellular signal-regulated kinase 1/2 [ERK 1/2]; c-Jun. NH2-terminal protein kinase [JNK]) and SB203580 (SB) were purchased from Cell Signaling Technology (Beverly, MA, USA). Inhibitory kappa-Bα (Iκ-Bα) monoclonal antibodies and a peroxidase-conjugated secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Anti-acetyl-histone H3 (Lys9) antibodies were purchased from Merck Milipore (Darmstadt, Germany). Pre-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) markers were purchased from Bio-Rad (Hercules, CA, USA). TRIzol reagent and polymerase chain reaction (PCR) kits were purchased from Invitrogen Corporation (Carlsbad, CA, USA).



Scheme 1. The structure of desoxo-narchinol-A.

2.3. Animal model of LPS-induced endotoxin shock

Female 6–8-week-old C57BL/6 mice weighing 15–20 g were purchased from Orient Bio (Sungnam, Kyungki-Do, South Korea). All animals were bred and housed in standard shoebox cages in a climate-controlled room with an ambient temperature of 23 ± 2 °C and a 12 h light–dark cycle for 7 days. Endotoxin shock was induced in mice by intraperitoneal injection of bacterial endotoxin (LPS from *E. coli* serotype O55:B5, 37.5 mg/kg). At 1 h after DN administration, LPS (37.5 mg/kg) was injected intraperitoneally. Survival was monitored for 120 h. Animal use and relevant experimental procedures were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. All experiments were approved by the Animal Care Committee of Wonkwang University.

2.4. Preparation of blood, liver and lung samples

DN (0.05, 0.1 and 0.5 mg/kg) was administered intraperitoneally to mice (n = 6 per group). One hour after DN administration, LPS (37.5 mg/kg) was injected intraperitoneally. Serum, liver, and lung samples were obtained from each mouse 3 h after LPS challenge and stored at −70 °C until use. Liver and lung tissues were fixed in 4% neutral buffered formaldehyde solution for 12 h, embedded in paraffin, and cut into 4-mm thick sections, which were stained with hematoxylin and eosin (H&E) to observe the morphological changes under a light microscope.

2.5. Messenger RNA (mRNA) expression

Total RNA was isolated from the mouse liver and lung using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and was subjected to reverse transcription using an ABI cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). TaqMan quantitative RT-PCR was performed using a StepOne Plus system according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA, USA). For each sample, triplicate test reactions and a control reaction lacking reverse transcriptase were analyzed for expression of the gene of interest, and the results were normalized to those of the "housekeeping" gene, hypoxanthine guanine phosphoribosyl-transferase (HPRT). Arbitrary expression units were calculated by division of the expression of the gene of interest by the expression of ribosomal protein HPRT mRNA. The commercial forward, reverse, and probe oligonucleotide primers for multiplex real-time TaqMan PCR were as follows: for mouse TNF-α (forward, 5′-TCT CTT CAA GGG ACA AGG CTG-3′; reverse, 5′-ATA GCA AAT CGG CTG ACG GT-3′; universal probe of ABI), for mouse IL-1β (forward, 5′-TTG ACG GAC CCC AAA AGA T-3′; reverse, 5′-GAA GCT GGA TGC TCT CAT CTG-3′; universal probe of ABI), and for mouse IL-6 (forward, 5′-TTC ATT CTC TTT GCT CTT GAA TTA GA-3′; reverse, 5′-GTC TGA CCT TTA GCT TCA AAT CCT-3′; universal probe of ABI).

2.6. Peritoneal macrophage culture

Thioglycollate (TG)-elicited peritoneal macrophages were harvested 4 days after intraperitoneal injection of 3 mL TG. Peritoneal lavage was performed using 8 mL of RPMI media supplemented with 10% heat-inactivated FBS. After incubation for 3 h, non-adherent peritoneal cells were removed and the medium was changed for adherent cells.

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