Journal of Ethnopharmacology 144 (2012) 109–117

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Achyranthes japonica exhibits anti-inflammatory effect via NF-κB suppression and HO-1 induction in macrophages

Soo Young Bang [a,1], Ji-Hee Kim [a,1], Hee-Young Kim [a], Young Ji Lee [a], Sun Young Park [a], Sang Joon Lee [b], YoungHee Kim [a,*]

[a] Department of Molecular Biology, College of Natural Sciences, Pusan National University, Busan 609-735, South Korea
[b] Department of Microbiology, College of Natural Sciences, Pusan National University, Busan 609-735, South Korea

ARTICLE INFO

Article history:
Received 22 March 2012
Received in revised form
7 August 2012
Accepted 25 August 2012
Available online 5 September 2012

Keywords:
Achyranthes japonica
Nitric oxide (NO)
Inducible nitric oxide synthase (iNOS)
Nuclear factor-kappaB (NF-κB)
Heme oxygenase-1 (HO-1)
NF-E2-related factor 2 (Nrf2)

ABSTRACT

Ethnopharmacological relevance: The roots of *Achyranthes japonica* Nakai have been used in traditional herbal medicine for the treatment of edema and arthritis in Korea.
Aim of the study: In this study, we investigated the molecular mechanism responsible for anti-inflammatory effects of the aqueous extract of *A. japonica* roots (AJ) in LPS-stimulated macrophages.
Materials and methods: Nitric oxide (NO) production and as inducible nitric oxide synthase (iNOS) expression were examined in TG-elicited peritoneal macrophages and RAW 264.7 cells. Cell viability was monitored by MTT assay. Protein and mRNA expressions were determined by Western blotting and RT-PCR, respectively. The activity of NF-κB and Nrf2 were examined by EMSA, immunocytochemistry or reporter assay.
Results: AJ inhibited LPS-induced NO secretion as well as iNOS expression, without affecting cell viability. Furthermore, AJ suppressed LPS-induced NF-κB activation, degradation of IκB-α, phosphorylation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. Further study demonstrated that AJ induced heme oxygenase-1 (HO-1) gene expression via nuclear translocation and transactivation of Nrf2. In addition, the inhibitory effects of AJ on iNOS expression were abrogated by small interfering RNA-mediated knock-down of HO-1.
Conclusions: These results suggest that AJ suppresses LPS-induced NO production and iNOS expression in macrophages through the inhibition of IκB/NF-κB and MAPK as well as the Nrf2-mediated HO-1 induction. These findings provide the scientific rationale for anti-inflammatory therapeutic use of *A. japonica* roots.

1. Introduction

Achyranthes japonica Nakai is a perennial herb and widely distributed throughout in Korea. Its roots have been called "Soe-moo-reup" in Korea, which means ox knee derived from their shape. They have been used in traditional medicine for the treatment of edema, arthritis, and delayed menses and as a contraceptive and abortifacient (Ahn, 2003). The constituents such as ecdysterone, inokosterone, and oleanolic acid have been identified from this plant (Ahn, 2003; Kim et al., 2008). A few biological activity of *A. japonica* has been reported; it inhibits platelet aggregation (Yun-Choi et al., 1985) and has anti-fungal (Kim et al., 2004c) and anti-inflammatory activities (Han et al., 1972). Oleanolic acid glycosides from *A. japonica* have anti-complement activity (Jung et al., 2012). However, little is known about the mechanism responsible for anti-inflammatory effects of *A. japonica* roots.

Nitric oxide (NO) is a free radical with multiple effects on various organ systems. The most prominent physiological actions of NO as a biological mediator include cGMP-dependent vasodilation, neural communication, host defense, inflammation, immune suppression and blood clotting (Moncada et al., 1991). NO is produced in physiological and pathophysiological conditions by NO synthase (NOS), and inducible NOS (iNOS) is induced by inflammatory cytokines and/or bacterial lipopolysaccharide (LPS) in various cell types including macrophages. A large amount of NO, particularly synthesized by iNOS, induces an inflammatory response to inhibit the growth of invading microorganisms and tumor cells. This strong inflammatory response to foreign cells could also cause further damage for the neighboring cells and tissues of the host (MacMicking et al., 1997). Therefore isozyme specific inhibitors of NOS are essential for therapeutic purposes and drugs that specifically inhibit iNOS could be

* Corresponding author. Tel.: +82 51 510 2526; fax: +82 51 513 9258.
E-mail address: yheekim@pusan.ac.kr (Y. Kim).
[1] These authors contributed equally to this work.

0378-8741/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2012.08.037

useful in treating diseases mediated by NO overproduction (Southan and Szabo, 1996).

NF-κB has been shown to play a major role in expression of the iNOS gene in response to LPS (Lowenstein et al., 1993; Xie et al., 1994). NF-κB is a heterodimeric transcription factor composed of p50 and p65 (RelA), but a variety of other Rel-containing dimers are also known to exist (Mercurio and Manning, 1999). In unstimulated cells, NFκB is present in the cytosol bound to the inhibitory protein I kappa B (IκB). In response to stimulation such as LPS, IκBs are rapidly ubiquitinated and degraded by 26S proteasome complex. The free NF-κB dimers translocate to the nucleus, bind with high affinity to specific sites in the promoter regions of target genes and stimulate their transcription.

Heme oxygenase-1 (HO-1) is an inducible enzyme that catalyzes the rate-limiting step in the oxidative degradation of cellular heme into carbon monoxide (CO), biliverdin, and free iron (Ryter et al., 2006). HO-1 and its enzymatic by-products provide a host defense mechanism that can protect the body against oxidative injury and also contributes to the anti-inflammatory activity of cells and tissues (Otterbein et al., 2000). In activated macrophages, HO-1 expression or CO treatment inhibits the production of the pro-inflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1β (Otterbein et al., 2000). Up-regulation of HO-1 expression or the administration of CO also suppresses the production of pro-inflammatory mediators such as nitric oxide (NO) and prostaglandin E2 (PGE2) (Suh et al., 2006). Moreover, an increasing number of therapeutic agents have been reported to induce HO-1 expression and exert their anti-inflammatory effects through HO-1 induction. These studies support beneficial effects of HO-1 that may serve as a therapeutic target in inflammatory diseases.

HO-1 is primarily regulated on the transcriptional level via signaling pathways involved in survival and stress responses in different cell types. Transcription factor NF-E2-related factor 2 (Nrf2) plays a central role for inducible expression of HO-1 (Srisook et al., 2005). In basal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and degraded by the ubiquitindependent 26S proteasome system (Itoh et al., 2010). Under activation, Nrf2 released from Keap1 inhibition, translocates to the nucleus, heterodimerizes with Maf, and binds antioxidant response elements (AREs) located in the promoter regions of many detoxifying/antioxidant genes, including HO-1 (Itoh et al., 2010; Motohashi et al., 2004).

In the present study, we investigated the effects of the aqueous extract of *A. japonica* roots (AJ) on LPS-induced inflammatory response (NO release) in macrophages and further explored the possible mechanisms. We examined the effect of *A. japonica* on NF-κB and HO-1 induction which have been known to regulate the expression of iNOS. Our results provide a molecular basis for understanding the inhibitory effects of *A. japonica* roots on endotoxin-mediated inflammation.

2. Materials and methods

2.1. Materials and preparation of extract

Cobalt protoporphyrin (CoPP) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Actinomycin D (Act. D) and cycloheximide (CHX) were purchased from Sigma-Aldrich Co. (St. Louis, MO). The dry roots of *A. japonica* were purchased from a local herb store, Kwang Myoung Dang (Busan, Korea) in April 2005. The roots of *A. japonica* were identified and authenticated by Professor W. S. Ko, College of Oriental Medicine, Dongeui University (Busan, Korea). The fingerprint chromatogram for the quality control of *A. japonica* was established by HPLC-DAD using ecdysterone (20-hydroxyecdysone, 20-HE) as a marker (Kim et al., 2008) (Fig. 1). A voucher specimen (number AJ-05-04) has been deposited at the Department of Molecular Biology, Pusan National University, Busan, Korea. The dry roots (300 g) were extracted with distilled water at 100 °C for 2 h. The extract was filtered through 0.45 μm filter and freeze-dried and kept at 4 °C. A yield of 31.74 g (10.58%) was obtained. The dried extract was dissolved in phosphate buffered saline (PBS) and filtered through 0.22 μm filter before use.

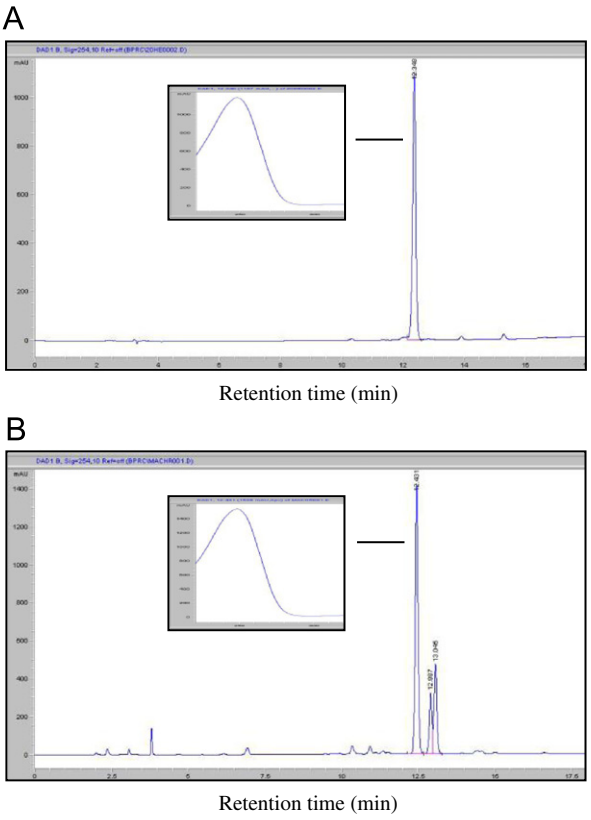

Fig. 1. HPLC chromatogram of 20-HE (A) and *A. japonica* (B). Inserts are UV spectrum of each peak.

2.2. Cell culture

C57BL/6 mice purchased from Dae Han Laboratory Animal Center (Korea) were used between 8 to 12 weeks of age (25–30 g). All animal studies were conducted in accordance with the principles and procedures in the Pusan National University Institutional Animal Care and Use Committee guidelines. Thioglycollate (TG) broth (Brewer, DIFCO, Detroit, MI)-elicited macrophages were harvested 3 day after intraperitoneal injection of 2.5 ml TG into mice and isolated as reported previously (Park et al., 2009). Murine macrophage RAW 264.7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with glutamine (1 mM) and 10% FBS at 37 °C in an atmosphere of 5% CO_2.

2.3. Measurement of nitrite concentration

NO synthesis in cell cultures was measured by a microplate assay method. To measure nitrite, 100 μl aliquots were removed from conditioned medium and incubated with an equal volume of the Griess reagent (1% sulfanilamide/0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride/2.5% H_3PO_4) at room temperature for