Journal of Ethnopharmacology 169 (2015) 210–218

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Astragaloside IV and cycloastragenol are equally effective in inhibition of endoplasmic reticulum stress-associated TXNIP/NLRP3 inflammasome activation in the endothelium

Yan Zhao [a], Qiang Li [b], Wenjun Zhao [a], Jia Li [c,d], Yan Sun [c,d], Kang Liu [c,d], Baolin Liu [c,d], Ning Zhang [a,*]

[a] Experiment Center for Science and Technology, Shanghai University of Traditional Chinese Medicine, Shanghai, China
[b] Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China
[c] State Key Laboratory of Natural Medicines, Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing, China
[d] Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicines, Nanjing, China

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form
14 March 2015
Accepted 18 April 2015
Available online 25 April 2015

Keywords:
Astragaloside IV
Cycloastragenol
Endothelial dysfunction
Endoplasmic reticulum stress
Inflammasome

ABSTRACT

Ethnopharmacological relevance: Astragaloside IV and cycloastragenol are present together in *Astragalus membranaceus* Moench (Fabaceae) and this study aims to simultaneously investigate their regulation of endothelial homeostasis in the setting of endoplasmic reticulum stress (ER stress).
Material and methods: We stimulated endothelial cells with palmitate (PA 100 μM) to evoked ROS-associated ER stress and observed the effects of astragaloside IV and cycloastragenol on thioredoxin-interacting protein (TXNIP) expression, NLRP3 inflammasome activation and mitochondrion-dependent apoptosis.
Results: Astragaloside IV and cycloastragenol inhibited ROS generation and attenuated ER stress inducer IRE1α phosphorylation, indicating the inhibition of ROS-associated ER stress. In response to ER stress, TXNIP expression increased, accompanied with NLRP3 induction and increased IL-1β and IL-6 production, but these alternations were reversed by treatment with astragaloside IV and cycloastragenol, demonstrating the inhibitory effects of astragaloside IV and cycloastragenol on TXNIP/NLRP3 inflammasome activation. Inflammasome activation led to mitochondrial cell death in endothelial cells, whereas astragaloside IV and cycloastragenol restored the loss of the mitochondrial membrane potential with inhibition of caspase-3 activity, and thereby protected cells from ER stress-induced apoptosis. Astragaloside IV and cycloastragenol enhanced AMPK phosphorylation and AMPK inhibitor compound C diminished their beneficial effects, indicative of the potential role of AMPK in their regulation.
Conclusions: Astragaloside IV and cycloastragenol suppressed ROS-associated ER stress and then inhibited TXNIP/NLRP3 inflammasome activation with regulation of AMPK activity, and thereby ameliorated endothelial dysfunction by inhibiting inflammation and reducing cell apoptosis. Simultaneous investigations further showed that astragaloside IV and cycloastragenol were equally effective in regulation of endothelial homeostasis.

1. Introduction

The endoplasmic reticulum (ER) is a cellular organelle that functions as a center for lipid synthesis and protein folding as well as calcium store regulation (Lin et al., 2008). In addition, it is also a major signal-transducing organelle that responds to alternations in cellular homeostasis (Xu et al., 2005). In fact, endoplasmic reticulum stress (ER stress) is an adaptive response to the accumulation of unfolded protein, and therefore, it is also termed as the unfolded protein response (UPR). When ER homeostasis is disrupted, the accumulation of unfolded protein aggregates lead to the activation of transmembrane sensors, including IRE-1α, which result in changes in gene expression and protein synthesis for the resolve of unfolded protein (Cox et al., 1993). Failure to adapt to increased unfolded proteins or aberrant ER stress is proposed to trigger inflammation and cell death (Xu et al., 2005; Hetz, 2012). In response to ER stress, the increased protein-folding promotes ROS generation and the accumulation of unfolded protein in ER leads to IRE-1α induction, which is important for integrating ER-stress signaling with inflammatory-response signaling (Zhang and Kaufman, 2008). ER stress-associated oxidative stress and inflammation are now thought to be fundamental in the pathogenesis

* Corresponding author. Tel.: +86 021 51322384; fax: +86 021 51322686.
E-mail address: ningzh18@yeah.net (N. Zhang).

http://dx.doi.org/10.1016/j.jep.2015.04.030
0378-8741/

of metabolic disorders, such as diabetes mellitus, obesity, and atherosclerosis (Zhang and Kaufman, 2008; Ozcan et al., 2004).

Oxidative stress and inflammation are implicated in ER stress, however thioredoxin-interacting protein (TXNIP)/NLRP3 inflammasome activation emerges as a key link among ER stress and inflammation and cell apoptosis (Zhou et al., 2010; Oslowski et al., 2012). TXNIP is a binding partner of reduced thioredoxin (TRX) and functions as a negative regulator of the TRX function (Nishiyama et al., 1999), while NLRP3 inflammasome is a functional complex responsible for immune responses through the maturation of pro-inflammatory cytokine IL-1β (Schroder and Tschopp, 2010). In response to ER stress, TXNIP dissociates from the binding to TRX and then induces NLRP3 inflammasome activation, which mediates inflammation and cell death through the processing of mature IL-1β in a manner caspase-1 dependent (Choi and Ryter, 2014), and the resultant production of IL-1β is required for inflammation and apoptosis in specialized cells (Oslowski et al., 2012).

Endothelial dysfunction is an early manifestation of cardiovascular diseases and accumulating evidence demonstrates the implication of ER stress in endothelial dysfunction. Though oxidative stress, inflammation and apoptosis occur simultaneously in endothelial dysfunction, ER stress emerges as a potential cause for these events. TXNIP is also expressed in the endothelium and its induction has been demonstrated to be involved in the initiation of inflammation (Perrone et al., 2009; Wang et al., 2012). A recent study further showed that TXNIP/NLRP3 inflammasome activation is required for endothelial inflammation and cell death in rats fed with high-fat diet (Mohamed et al., 2014). Although the link between ER stress and NLRP3 inflammasome activation is not fully elucidated in the endothelium, above-mentioned evidence suggests that TXNIP/NLRP3 inflammasome activation plays an important role in endothelial dysfunction.

Astragaloside IV is a saponin and serves as the predominant constituent of *Astragalus membranaceus* (Kwon and Park, 2012). In China, *A. membranaceus* has been considered to be a health-enhancing herbal used for the treatment of metabolic and cardiovascular disorders, including diabetes and atherosclerosis. Although cycloastragenol is also present in the flowers and roots of *A. membranaceus* (Verotta et al., 2002), it can be generated from parent astragaloside IV through hydrolysis by intestinal bacteria (Zhou et al., 2012) (Fig. 1). Astragaloside IV inhibits inflammatory and oxidative responses (Li et al., 2013; Gui et al., 2013a), and these actions have been shown to be implicated in the protection of cells from apoptosis (Gui et al., 2012). Moreover, clinical application and published studies demonstrate its beneficial effects on regulation of endothelial functions (Zhang et al., 2006, 2007, 2011). Despite these studies show the actions of astragaloside IV in inhibition of inflammation and regulation of vasorelaxation in the endothelium, the potential molecular targets or pathways remain to be elucidated. The bioactivity of astragaloside IV has been well documented, but little is known about the action of cycloastragenol. Because cycloastragenol is a microbial transformation of astragaloside IV and both astragaloside IV and cycloastragenol are present together in the blood after oral administration of *A. membranaceus* (Zhou et al., 2012), it is tempting to know whether cycloastragenol demonstrates a similar or different action with astragaloside IV in regulation of the endothelial function, especially in the setting of ER stress. To address these issues, this study aims to investigate the effects of astragaloside IV and cycloastragenol simultaneously on the endothelial homeostasis under ER stress conditions. Our work demonstrated that astragaloside IV and cycloastragenol ameliorated endothelial dysfunction by suppression of TXNIP/NLRP3 inflammasome activation with regulation of AMPK activity and further showed that their actions were equally effective. Our findings provide novel mechanistic insights regarding the protective effects of astragaloside IV and cycloastragenol on endothelial homeostasis. Because astragaloside IV as well as cycloastragenol are the main active ingredients of *A. membranaceus* in regulating endothelial homeostasis and responsible for the action of *A. membranaceus* in the clinic, we expect this new knowledge would contribute to designing novel strategies for their application in the management of diabetes and cardiovascular diseases.

2. Materials and methods

2.1. Reagents

Astragaloside IV (purity > 98%) was purchased from Shanghai Forever Biotech Co., Ltd. (Shanghai, China). Product ID: E-0146. Cycloastragenol (purity > 97%) was obtained from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). Product ID: E-0646. AICA riboside (AICAR) and mitoquinone mesylate (Mito Q) were obtained from Beyotime Institute of Biotechnology (Shanghai, China). Compound C was provided by Sigma (St. Louis, MO, USA). These agents were dissolved in dimethyl sulfoxide (DMSO) and the final concentration was less than 0.01% (v/v). Palmitate (PA) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and dissolved in ethyl alcohol as a stock solution and then was further diluted with medium containing 10% of bovine serum albumin before use. Enhanced chemiluminescence (ECL) was obtained from Beyotime Institute of Biotechnology (Shanghai, China). The following items were purchased from the cited commercial sources: anti-phospho-AMPKα (T172) (2531s) and anti-AMPKα (2532s), Cell Signaling Technology (Beverly, MA, USA); anti-phospho-IRE1α

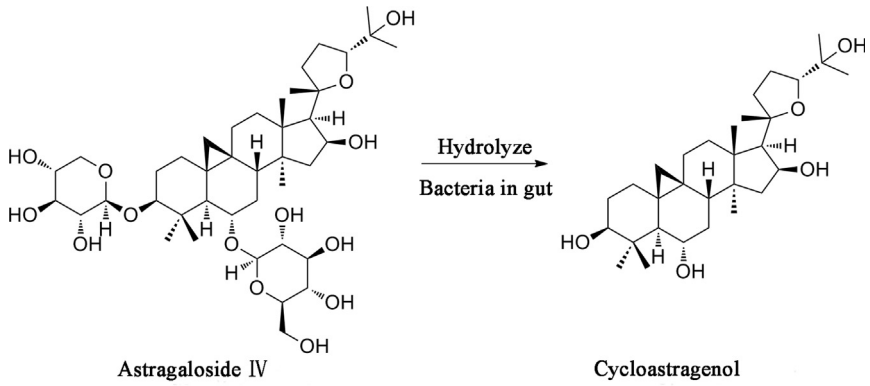

Fig. 1. Structures of astragaloside IV and cycloastragenol. As an aglycone, cycloastragenol can be generated from parent astragaloside IV through hydrolysis by intestinal bacteria after oral administration.