Osthole Regulates Inflammatory Mediator Expression through Modulating NF-κB, Mitogen-Activated Protein Kinases, Protein Kinase C, and Reactive Oxygen Species

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Osthole, a coumarin compound, has been reported to exhibit various biological activities; however the cellular mechanism of its immune modulating activity has not yet been fully addressed. In this study we isolated osthole from the seeds of Cnidium monnieri and demonstrated that osthole inhibited TNF-α, NO and COX-2 expression in LPS-stimulated macrophages, without reducing the expression of IL-6. Furthermore, the phosphorylation of p38, JNK1/2, PKC-α and PKC-ε induced by LPS was inhibited by osthole; however, the phosphorylation of ERK1/2 and PKC-δ was not reduced by osthole. Osthole also inhibited NF-κB activation and ROS release in LPS-stimulated macrophages. Our current results indicated that osthole is the major anti-inflammatory ingredient of Cnidium monnieri seed ethanol extract.

KEYWORDS: Osthole; LPS; inflammation; signaling

INTRODUCTION

Cnidium monnieri Cuss. is not only a traditional Chinese herb but also an economically important agricultural product via artificial planting, especially in China in recent years. Osthole (7-methoxy-8-isopentenoxycoumarin), a coumarin compound isolated from the seeds of C. monnieri, exhibits significant bioactivities including induction of apoptosis in HER2-overexpressing breast cancer cells (1); inhibition of voltage-gated Na⁺ channels in mouse neuroblastoma N2A cells (2); inhibition of rat vascular smooth muscle cell proliferation (3); suppression of the secretion of hepatitis B virus through increasing the glycosylation of hepatitis B surface antigen which are important steps for the viral particle maturation (4); inhibition of contact dermatitis in experimental animals (5); and inhibition of cytokine expression in rat peritoneal cells and human peripheral blood mononuclear cells (6). However, the molecular mechanism of osthole-mediated downregulation of tumor necrosis factor-alpha (TNF-α), nitric oxide (NO) and cyclooxygenase-2 (COX-2) expression in macrophages is unclear.

The innate immunity of mammals is triggered by pathogen-associated molecular patterns that are shared by groups of different microbial pathogens; these are recognized by Toll-like receptors (TLRs) expressed on the cell surface of macrophages (7). Lipopolysaccharide (LPS), one of the most important pathogen-associated molecular patterns, activates macrophages by binding to TLR4, followed by stimulating nuclear transcription factor kappa-B (NF-κB) activation. This leads to the production of proinflammatory mediators from macrophages, including TNF-α, interleukin-1β (IL-1β), interleukin-6 (IL-6) and NO (8). Protein kinase C (PKC) is one of the signaling molecules in an LPS-mediated inflammatory response, and regulates a downstream signal transduction cascade via modulation of the mitogen-activated protein kinase (MAPK) pathways, such as extracellular signal regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase 1/2 (JNK1/2) and p38 MAP kinase (9). Recently, the development of potential therapeutic approaches to modulate inflammatory disease has become ever more popular and important. These therapeutic approaches include inhibition of proinflammatory mediator production (10).

In our previous study we found that ethanol extract of C. monnieri seeds inhibited cytokine production in LPS-stimulated macrophages. In addition, Zimecki et al. reported that osthole inhibited concanavalin A- and pokeweed mitogen-induced mouse splenocyte proliferation and inhibited TNF-α production in rat peritoneal cells and human peripheral blood mononuclear cells (6). This finding promoted us to isolate osthole, the major component of the ethanol extract of C. monnieri seeds, and dissect its anti-inflammatory mechanisms in macrophages. We demonstrated