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Caffeic acid phenethyl ester protects mice from lethal endotoxin shock and inhibits lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in RAW 264.7 macrophages via the p38/ERK and NF-κB pathways

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ABSTRACT

Caffeic acid phenethyl ester has been shown to have anti-inflammatory and anti-cancer effects. We examined the effects of caffeic acid phenethyl ester on lipopolysaccharide-induced production of nitric oxide and prostaglandin E_2, and expression of inducible nitric oxide synthase and cyclooxygenase-2 in RAW 264.7 macrophages. We also investigated the effects of caffeic acid phenethyl ester on lipopolysaccharide-induced septic shock in mice. Our results indicate that caffeic acid phenethyl ester inhibits lipopolysaccharide-induced nitric oxide and prostaglandin E_2 production in a concentration-dependent manner and inhibits inducible nitric oxide synthase and cyclooxygenase-2 in RAW 264.7 cells, without significant cytotoxicity. To further examine the mechanism responsible for the inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by caffeic acid phenethyl ester, we examined the effect of caffeic acid phenethyl ester on lipopolysaccharide-induced nuclear factor-κB activation and the phosphorylation of mitogen-activated protein kinases. Caffeic acid phenethyl ester treatment significantly reduced nuclear factor-κB translocation and DNA-binding in lipopolysaccharide-stimulated RAW 264.7 cells. This effect was mediated through the inhibition of the degradation of inhibitor κB and by inhibition of both p38 mitogen-activated protein kinase and extracellular signal-regulated kinase phosphorylation, at least in part by inhibiting the generation of reactive oxygen species. Furthermore, caffeic acid phenethyl ester rescued C57BL/6 mice from lethal lipopolysaccharide-induced septic shock, while decreasing serum levels of tumor necrosis factor-α and interleukin-1β. Collectively, these results suggest that caffeic acid phenethyl ester suppresses

Abbreviations: CAPE, caffeic acid phenethyl ester; iNOS, inducible nitric oxide synthase; PGE_2, prostaglandin E_2; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; NF-κB, nuclear factor-κB; IκB, inhibitory factor of NF-kB; MAPK, mitogen-activated protein kinases; ROS, reactive oxygen species; MOF, multiple organ failure.

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the induction of cytokines by lipopolysaccharide, as well as inducible nitric oxide synthase and cyclooxygenase-2 expression, by blocking nuclear factor-κB and p38/ERK activation. These findings provide mechanistic insights into the anti-inflammatory and chemopreventive actions of caffeic acid phenethyl ester in macrophages.

1. Introduction

Caffeic acid phenethyl ester (CAPE), a potent flavonoid anti-oxidant, is an active component in propolis. It has strong anti-viral, anti-tumoral, anti-inflammatory, anti-oxidant, neuroprotective, anti-atherosclerotic and immunomodulatory properties in diverse systems (Orsolic et al., 2005). CAPE is also a potent and specific inhibitor of nuclear transcription factor-κB (NF-κB) activation (Natarajan et al., 1996). NF-κB is normally present in the cytosol and exists as an inactive complex with a class of inhibitory proteins, known as inhibitor κB (IκB) proteins. Following an inflammatory stimulus, the phosphorylation of IκB triggers its degradation and the translocation of NF-κB to the nucleus, where it binds to promoter regions and induces the expression of a wide variety of genes involved in inflammation, including those encoding cytokines (such as IL-1, IL-6 and TNF-α), enzymes (including nitric oxide synthase), adhesion molecules and acute-phase proteins. Because of its ubiquitous role in the pathogenesis of inflammatory gene expression, NF-κB is a current target for the treatment of various diseases (Barnes and Karin, 1997; Makarov, 2000; Renard and Raes, 1999).

Nitric oxide has been shown to be an important regulatory molecule in diverse physiological functions, including vasodilation, neural communication and host defense (MacMicking et al., 1997; Mitchell et al., 1995). In mammalian cells, nitric oxide (NO) is synthesized by three different isoforms of nitric oxide synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Importantly, iNOS is highly expressed in lipopolysaccharide (LPS)-activated macrophages and contributes to the pathogenesis of septic shock (Petros et al., 1991; Thiemermann, 1997). The promoter region of the murine gene encoding iNOS contains NF-κB binding motifs. It has been reported that binding of NF-κB to the NF-κB sites upstream of the iNOS promoter plays an important role in the LPS-induced upregulation of the iNOS gene. Cyclooxygenase (COX) is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandin H2, a precursor for a variety of biologically active mediators, such as prostaglandin E_2 (PGE_2), prostacyclin and thromboxane A2 (Picot et al., 1994; Hawkey, 1999). Two forms of COX have been identified: cyclooxygenase-1 (COX-1), a constitutive cyclooxygenase, and cyclooxygenase-2 (COX-2), which is induced in response to many stimulants and is activated at sites of inflammation (Mitchell et al., 1995; Smith et al., 1996). COX-2 is rapidly produced in macrophages and endothelial cells in response to proinflammatory cytokines and may be responsible for the edema and vasodilation associated with inflammation. It is well known that inflammatory mediators, including NO and COX-2, are responsible for the symptoms of many inflammatory diseases, such as rheumatoid arthritis, chronic hepatitis and pulmonary fibrosis (Isomaki and Punnonen, 1997; Tilg et al., 1992; Coker and Laurent, 1998). Thus, inhibition of these inflammatory mediators is an important target in the treatment of inflammatory diseases.

Septic shock is a severe inflammatory response that is triggered by systemic infection and is characterized by hypoperfusion of major organs, leading to multiple organ failure (MOF), shock and death. The pathogenesis of sepsis involves a progressive and dynamic expansion of a systemic inflammatory response to bacterial infection (Glauser, 1996). LPS, an integral part of the outer membrane of Gram-negative bacteria, is a major pathogenic factor in septic shock. Many treatment strategies for this condition have been developed, but the mortality rate has not improved substantially (Bone et al., 1995; Giroi et al., 1997).

LPS causes phosphorylation of p38 mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK)-1/2 and c-Jun NH_2-terminal kinase (JNK), leading to the activation of NF-κB in macrophages. Moreover, three well-defined MAPKs, ERK-1/2, p38 MAPK and JNK/SAPK, have been implicated in the transcriptional regulation of the iNOS and COX-2 genes. Collectively, the results of these studies suggest that MAPK activation significantly regulates NO and PGE_2 production by controlling the activation of NF-κB.

In this study, we investigated the effects and mechanisms of action of CAPE (Fig. 1) on endotoxin-stimulated proinflammatory mediators, and the findings suggest that CAPE has therapeutic potential against inflammatory diseases, including sepsis and endotoxemia.

2. Materials and methods

2.1. Materials

CAPE, LPS (*Escherichia coli* 026:B6), *p*-nitrophenyl phosphate and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO). RT-PCR reagents were purchased from Promega (Madison, WI). Reagents for Lightshift chemiluminescent electrophoretic mobility shift assays, nuclear and cytoplasmic extraction and biotin 3′ end labeling were purchased from Pierce (Rockford, IL). Specific antibodies against iNOS, COX-2 and p65 were purchased from

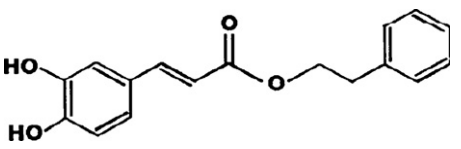

Fig. 1. Structure of caffeic acid phenethyl ester (2-cyclohexylethyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate).