Anti-inflammatory activity of *Camellia japonica* oil

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**INTRODUCTION**

Inflammation is the body’s response to cellular injury. It is a diverse process that is mediated by inflammatory or immune cells. Among these cells, macrophages play a central role in managing the overproduction of pro-inflammatory cytokines and inflammatory mediators. Among its mediators, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) have shown to be important enzymes that regulate inflammatory responses. Both iNOS and COX-2 are inducible enzymes that mediate similar pathological processes (1). NO is produced by nitric oxide synthase (NOS), which converts L-arginine to L-citrulline (2). Once expressed, iNOS synthesizes large amounts of NO, which has both regulatory and detrimental effects (3, 4). During the inflammation response, NO overproduction may become cytotoxic (5).

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*Camellia japonica* oil (CJ oil) has been used traditionally in East Asia to nourish and soothe the skin as well as help restore the elasticity of skin. CJ oil has also been used on all types of bleeding instances. However, little is known about its anti-inflammatory effects. Therefore, the anti-inflammatory effects of CJ oil and its mechanisms of action were investigated. CJ oil inhibited LPS-induced production of NO, PGE₂, and TNF-α in RAW264.7 cells. In addition, expression of COX-2 and iNOS genes was reduced. To evaluate the mechanism of the anti-inflammatory activity of CJ oil, LPS-induced activation of AP-1 and NF-κB promoters was found to be significantly reduced by CJ oil. LPS-induced phosphorylation of IkBα, ERK, p38, and JNK was also attenuated. Our results indicate that CJ oil exerts anti-inflammatory effects by downregulating the expression of iNOS and COX-2 genes through inhibition of NF-κB and AP-1 signaling. [BMB reports 2012; 45(3): 177-182]

COX converts arachidonic acid to prostaglandins (PGs), and like NOS, COX exists in two isoforms, COX-1 and COX-2. COX-1 and COX-2 catalyze the rate-limiting step in the production of PGs, which are bioactive compounds involved in processes such as fever and sensitivity to pain (6). COX-1 is ubiquitously and constitutively expressed, whereas COX-2 is highly inducible and generally present at very low levels, unless increased by one of many types of stimuli. COX-2 is also regulated at the post-transcriptional and enzymatic levels.

The transcriptional mediator nuclear factor-kappaB (NF-κB) plays a major role in regulating inflammatory responses by increasing the level of cytokines, chemokines, growth factors, and cell adhesion molecules (7). The activation of NF-κB results in the expression of these pro-inflammatory genes (8). These include the transcription of various inflammatory cytokines and TNF-α (9), as well as genes encoding COX-2 and iNOS (10). In addition, the transcription factor activator protein-1 (AP-1) regulates transcriptional genes of inflammatory responses (11). Mitogen-activated protein kinase (MAPKs) can phosphorylate transcription factors such as NF-κB and AP-1, which leads to the expression of pro-inflammatory mediators and cytokines of extracellular stimuli (12).

*Camellia japonica* is a native plant grown in Jeju Island which is called the island of camellia trees. CJ oil has a long history of use as a cosmetic protectant to keep skin and hair healthy and as a soothing agent. It has been reported that *Camellia japonica* possesses a variety of biological activities, including antibacterial activity (13), inhibitor of human immunodeficiency virus type 1 protease (14), Epstein-Barr virus inhibitor (15), anti-metastasis activity (16), antioxidant activity (17, 18), inhibitor of human type I pro-collagen production (19), and anti-allergic responses (20). Despite its wide spread use, there have been no studies that have examined the effects of CJ oil on inflammation-associated gene expression.

Therefore, in this study we characterized the inhibitory effects and mechanisms of CJ oil against inflammatory signals and demonstrated that CJ oil inhibits LPS-induced inflammatory reactions through inactivation of AP-1 and NF-κB pathway in RAW264.7 cells.
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Fig. 1. CJ oil inhibited NO, PGE\(_2\), and TNF-\(\alpha\) production in RAW264.7 cells. (a) NO production, (b) PGE\(_2\) production, (c) TNF-\(\alpha\) production, (d) Cell viability. Data are expressed as means ± S.D. Data points with different letters represent values that are significantly different from each other at the P = 0.05 level. Dm: Dexamethasone.

RESULTS

CJ oil reduced LPS-induced production of NO, PGE\(_2\), and TNF-\(\alpha\) in RAW264.7 cells

Pro-inflammatory mediators such as NO, PGE\(_2\), and TNF-\(\alpha\) play very important roles in the inflammatory response. To investigate the effect of CJ oil on the production of these mediators, the levels of secreted NO, PGE\(_2\), and TNF-\(\alpha\) were measured. As shown in Fig 1a, b, and c, LPS-induced production of NO, PGE\(_2\), and TNF-\(\alpha\) was significantly reduced by CJ oil in a concentration-dependent manner. Dexamethasone was used as a positive control. To exclude the possibility that this effect was due to the cytotoxic effect of CJ oil, an MTT assay was performed. Cytotoxic effects of CJ oil were not observed at the tested concentrations (Fig. 1d). These results indicate that CJ oil produces anti-inflammatory effects.

LPS-induced expression of iNOS and COX-2 genes was attenuated by CJ oil in RAW264.7 cells

NO and iNOS mediate inflammatory responses (4). COX-2 is also a key enzyme that regulates the production of prostaglandins, which are central mediators of inflammation (21). Therefore, to investigate the effects of CJ oil on the expression of iNOS and COX-2 genes, a luciferase reporter and Western blot assays for iNOS and COX-2 were performed. As shown in Fig 2a and b, LPS-induced activation of iNOS and COX-2 promoters was significantly inhibited by CJ oil. Similarly, CJ oil reduced LPS-induced expression of iNOS and COX-2 proteins (Fig. 2c and d), suggesting that CJ oil exerts anti-inflammatory effects by down-regulating the expression of iNOS and COX-2 genes.

CJ oil effects were mediated by inhibition of NF-κB and AP-1 (ERK, p38, and JNK) activation

NF-κB and AP-1 are important transcriptional factors that regulate the expression of the iNOS (22) and COX-2 (23) gene. Thus, we investigated the role of these transcription factors in the CJ oil-induced expression of iNOS and COX-2 genes. To this end, luciferase reporter assays for AP-1 and NF-κB promoters were performed in RAW264.7 cells. In this study, CJ oil suppressed LPS-induced activation of AP-1 and NF-κB promoters (Fig. 3a and b), suggesting that the effects of the CJ oil are dependent on AP-1 and NF-κB signaling. These results were further confirmed by Western blot for the phosphorylated forms of MAPKs and IκBα. As shown in Fig. 4, CJ oil suppressed LPS-induced phosphorylation of ERK, p38, and JNK. In addition, LPS-induced phosphorylation of IκBα was reduced by CJ oil. These results indicate that CJ oil reduces expression of iNOS and COX-2 genes via inhibition of NF-κB and AP-1