Cafestol, a coffee-specific diterpene, is a novel ERK inhibitor with AP-1-targeted inhibition of PGE_2 production in LPS-activated macrophages

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Objectives

Coffee is a most popular beverage in the world with various nutritional benefits. Diterpene cafestol, one of major components in coffee, contributes to its beneficial effects through displaying various biological activities such as chemopreventive, anti-tumorigenic, hepatoprotective, anti-oxidative and anti-inflammatoy effects. In this study, we examined exact molecular mechanism of anti-inflammatory activity by cafestol in terms of prostaglandin E_2 (PGE_2) production, a critical factor involved in inflammatory responses.

Materials and Methods

◦ Materials

Cafestol (purity: 99%), phorbol-12-myristate-13-acetate (PMA), and lipopolysaccharide (LPS, E. coli 0111:B4) were purchased from Sigma (St. Louis, MO). LY294002, wortmannin, U0126, SP600125, and SB203580 were obtained from Calbiochem (La Jolla, CA). Fetal bovine serum (FBS) was obtained from GIBCO (Grand Island, NY). All other chemicals were Sigma grade.

○ Methods

To do these experiments, anti-inflammatory effects such as PGE2 production, nitric oxide (NO) production, MTT assay, Luciferase Reporter Gene Activity Assay, RT-PCR and Immunoblotting were tested according to previous methods using murine macrophage cell line and Human embryonic kidney cell line.

Results

Cafestol inhibited both PGE_2 production and the mRNA expression of cyclooxygenase (COX)-2 from LPS-treated RAW264.7 cells. Interestingly, this compound strongly diminished the translocation of c-Jun into nucleus and AP-1-mediated luciferase activity. According to direct kinase assay, it was found that cafestol can act as an inhibitor to ERK2 but not ERK1 and MEK1. Therefore, our data suggest that cafestol may be a novel ERK inhibitor with AP-1-targeted inhibitory property on PGE_2 production in LPS-activated RAW264.7 cells.

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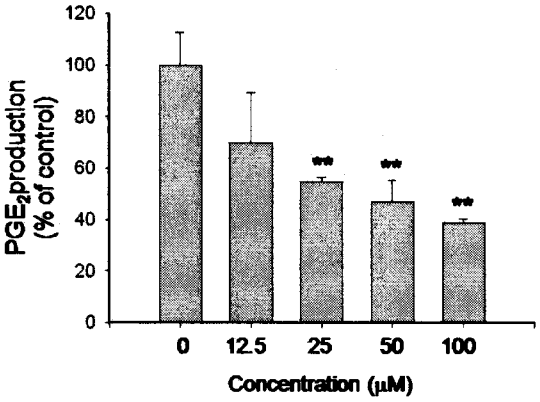
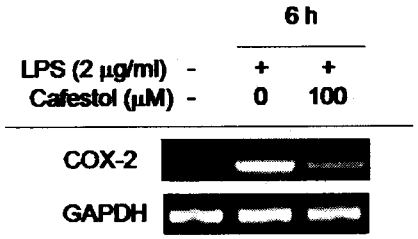
Fig.1 AP-1-targeted inhibitory effects of cafestol on PGE_2 production in LPS-activated RAW264.7 cells.

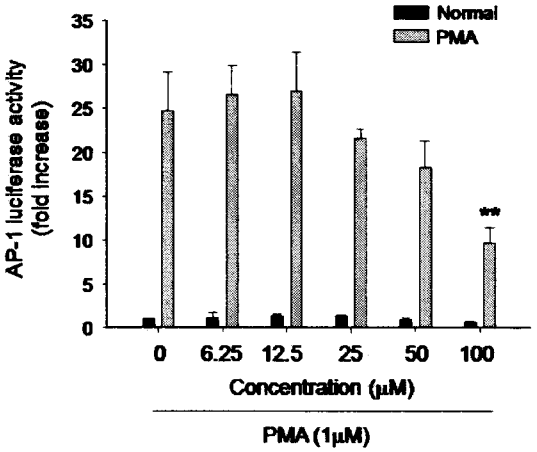
(A) PGE_2 production



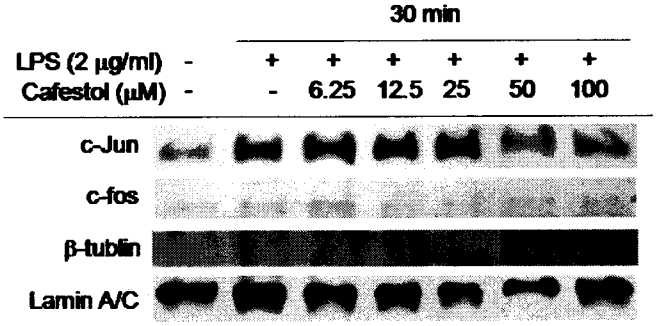
(B) mRNA expression level of COX-2



(C) AP-1-mediated luciferase activity



(D) Transcription factor level in Nuclear fraction



(E) Direct MAPK kinase activity