Suppressive effects of the kahweol and cafestol on cyclooxygenase-2 expression in macrophages

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Abstract **Inducible cyclooxygenase-2 (COX-2) has been suggested to play a role in the processes of inflammation and carcinogenesis. Recent studies have shown the chemoprotective effects of kahweol and cafestol, which are coffee-specific diterpenes. This study investigated the effects of kahweol and cafestol on the expression of COX-2 in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. Kahweol and cafestol significantly suppressed the LPS-induced production of prostaglandin E_2, COX-2 protein and mRNA expression, and COX-2 promoter activity in a dose-dependent manner. Furthermore, kahweol blocked the LPS-induced activation of NF-κB by preventing IκB degradation and inhibiting IκB kinase activity. These results will provide new insights into the anti-inflammatory and anti-carcinogenic properties of kahweol and cafestol.**

Keywords: Kahweol; Cafestol; Cyclooxygenase-2; NF-κB; Macrophage

1. Introduction

Cyclooxygenase (COX) catalyzes the synthesis of prostaglandins (PGs) from arachidonic acid. Two isozymes, COX-1 and COX-2, have been identified but are encoded by separate genes. The COX-1 isozyme is believed to be a housekeeping protein in most tissues and appears to catalyze the synthesis of PGs for normal physiological functions. In contrast, COX-2 is not present under normal physiological conditions but is rapidly induced in various cell types by tumor promoters, growth factors, cytokines and mitogens [1,2]. Many cell types associated with inflammation, such as macrophages, endothelial cells and fibroblasts, express the COX-2 gene upon induction [1]. It is well established that COX-2 is important in carcinogenesis, and is over-expressed in transformed cells as well as in various forms of cancer [1,2]. Because the targeted inhibition of COX-2 is a promising approach to inhibiting inflammation and carcinogenesis as well as to prevent cancer, various chemopreventive strategies have focused on inhibitors of the COX-2 enzyme activity. An equally important strategy may be to identify the compounds that suppress the signaling pathways that regulate COX-2 expression [3,4].

COX-2 is an early gene expressed in response to many cytokines. Its transcriptional regulation is, at least in part, under the control of the transcription factor nuclear factor-κB (NF-κB) [5]. In macrophages, LPS activates NF-κB, eventually induces the expression of many immediate early genes [5]. The presence of a *cis*-acting NF-κB element has been demonstrated in the 5′-flanking regions of the COX-2 gene [5,6]. NF-κB activation is induced by a cascade of events leading to the activation of the inhibitor κB (IκB) kinase (IKK), which phosphorylates IκB, leading to its degradation and finally resulting in the translocation of NF-κB to the nucleus [6,7]. Because NF-κB plays a key role in regulating the genes involved in the initiation of the immune, acute phase, and inflammatory responses, there is growing interest in modulating its activity. Therefore, the pathways leading to NF-κB activation are frequent targets for a variety of anti-inflammatory drugs [6].

It is increasingly being acknowledged that foods and beverages contain non-nutritional constituents that may have beneficial health effects, such as anti-inflammatory and anti-carcinogenic properties [8]. Kahweol and its dehydro derivative, cafestol (Fig. 1), are two diterpenes that are present in considerable quantities in coffee beans, as well as in the final, unfiltered beverage, e.g., in Turkish or Scandinavian style coffees [9]. They have been shown to possess both adverse and chemoprotective properties [10,11]. It is well known that both kahweol and cafestol increase the blood cholesterol level in both human and animal models [10]. However, animal studies have shown that kahweol and cafestol offer some protection against the action of well-known carcinogens [12,13]. In line with these observations, there is epidemiological evidence in humans that the consumption of coffee with a high amount of kahweol and cafestol is associated with a lower rate of colon cancer [14]. The chemoprotective effects of kahweol and cafestol have thus far been primarily related to the beneficial modifications of the xenobiotic metabolism. Such effects include the reduced activation of mutagens/carcinogens, e.g., via the inhibition of the cytochrome P450 enzymes [12], as well as their enhanced detoxification, e.g., via the induction of carcinogen-detoxifying enzyme systems such as glutathione *S*-transferase and UDP-glucuronosyl transferase [11,15].

Excessive prostaglandin E_2 (PGE_2) production by COX-2 in stimulated inflammatory cells is believed to be a causative factor associated with cellular injury in inflammatory disease. Therefore, compounds inhibiting COX-2 activity or its transcriptional activity might have anti-inflammatory or cancer chemopreventive applications. This study investigated the

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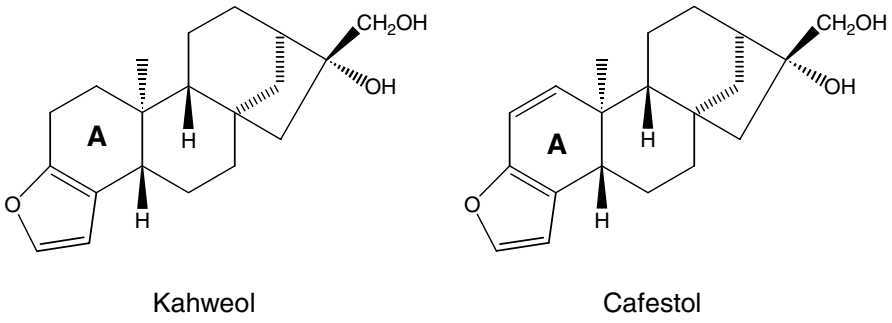

Fig. 1. Chemical structures of kahweol and cafestol. The arrows indicate the chemical differences between the two compounds; a double bond in kahweol is lacking in cafestol.

effects of kahweol and cafestol on the COX-2 expression level in murine macrophages. Herein, we show for the first time that kahweol and cafestol suppress the activation of COX-2 gene expression via NF-κB inhibition by targeting the IKK complex.

2. Materials and methods

2.1. Chemicals and materials

The chemicals and cell culture materials used in this study were obtained from the following sources: kahweol acetate, cafestol acetate, and *Escherichia coli* 0111:B4 lipopolysaccharide (LPS) from Sigma Co.; MTT-based colorimetric assay kit from Roche Co.; prostaglandin E_2 (PGE_2) and enzyme immunoassay reagents for the PGE_2 assays from Cayman Co.; LipofectAMINE Plus, RPMI 1640 medium, fetal bovine serum (FBS), and penicillin-streptomycin solution from Life Technologies, Inc.; pGL3-4κB-Luc and the luciferase assay system from Promega; pCMV-β-gal from Clonetech; GST-IκBα and antibodies to COX-2, COX-1, β-actin, IKKβ, IκBα, and the phosphorylated form of IκBα (Ser 32) from Santa Cruz Biotechnology, Inc.; Western blotting detection reagents (ECL) from Amersham Pharmacia Biotech.; the other chemicals were of the highest commercial grade available.

2.2. Cell culture and cell viability assay

The mouse macrophage cell line, RAW 264.7 cells, was obtained from the American Type Culture Collection (Bethesda, MD), and grown in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 humidified incubator. Kahweol acetate and cafestol acetate were dissolved in dimethyl sulfoxide and added directly to the culture media. The control cells were treated with the solvents only, the final concentration of which never exceeded 0.1%, which is a concentration that did not have any noticeable effect on the assay systems. The cell viability was assessed using a MTT assay according to the manufacturer's instructions.

2.3. Determination of PGE_2 production

The cells were incubated with the chemicals and/or LPS (0.5 μg/ml). After incubating the cells for 24 h, the culture medium was collected and the level of PGE_2 released into culture media was measured using a specific enzyme immunoassay according to the manufacturer's instructions.

2.4. Immunoblot analysis

The cells were cultured with the chemicals and/or LPS (0.5 μg/ml) for 24 h and equal amounts of the total cellular protein (50 μg) were resolved by 10% SDS–PAGE and transferred onto polyvinylidene difluoride membranes. After blocking, the membranes were incubated with COX-2 polyclonal antiserum, COX-1 polyclonal antiserum or monoclonal anti-β-actin. The secondary antibody to IgG conjugated to horseradish peroxidase was used. The blots were probed with the ECL Western blot detection system according to the manufacturer's instructions.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

The cells were cultured with the chemicals and/or LPS (0.5 μg/ml) for 3 h. The total cellular RNA was isolated using an acidic phenol extraction procedure. cDNA synthesis, semiquantitative RT-PCR for COX-1, COX-2, and β-actin mRNA, and the analysis of the results were all performed as previously described [16].

2.6. Transient transfection and luciferase and β-galactosidase assays

The cells were transiently co-transfected with the plasmids (the COX-2 promoter construct (−327/+59) and its NF-κB mutant constructs (designated KBM, −223/−214) have been described previously [17], which were generous gifts from Dr. Tadashi Tanabe and Chieko Yokoyama (National Cardiovascular Center Research Institute, Osaka, Japan), pGL3-4κB-Luc, and pCMV-β-gal) using LipofectAMINE Plus according to the manufacturer's protocol. After 18 h, the cells were treated with the chemicals and/or LPS (0.5 μg/ml) for 12 h, which were then lysed. The luciferase and β-galactosidase activity were determined as described previously [18]. The luciferase activity was normalized with respect to the β-galactosidase activity and was expressed relative to the activity of the LPS group.

2.7. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as previously described [18]. Two double-stranded deoxyoligonucleotides containing the NF-κB binding site (5′-CAGA**GGGGACTTTCC**GAGAG-3′, bold and underlined indicates NF-κB core consensus sequences) were end-labeled with [γ-^{32}P]dATP. Nuclear extracts (5 μg) were incubated with 2 μg of poly(dI–dC) and the ^{32}P-labeled DNA probe in binding buffer (100 mM NaCl, 30 mM HEPES, 1.5 mM $MgCl_2$, 0.3 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml concentration each of aprotinin and leupeptin) for 10 min on ice. DNA was separated from the free probe using a 4.8% polyacrylamide gel in 0.5× TBE buffer (44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA). Following electrophoresis, the gel was dried and subjected to autoradiography.

2.8. IκBα degradation and IKK assay

The cytoplasmic extracts were prepared from the cells treated with the chemicals and LPS (0.5 μg/ml) for 30 min. The extracts were then resolved on 10% SDS–PAGE and analyzed by immunoblotting using an antibody against IκBα, as described above. For the IKK assay, the cells were treated with the chemicals for 30 min, and equal amounts of the total cellular protein (800 μg) were immunoprecipitated with the IKKβ antibody and protein A/G-PLUS agarose for 12 h at 4 °C. The kinase assay was carried out in a kinase buffer containing 5 μM cold ATP, 10 μCi [γ-^{32}P]ATP (5000 Ci/mmol) and 1 μg of the GST-IκBα fusion protein as a substrate, and incubated for 20 min at 25 °C. The reaction was quenched by adding the Laemmli buffer followed by boiling for 5 min. The samples were subjected to 10% SDS–PAGE, transferred to polyvinylidene difluoride membranes, analyzed by autoradiography, and finally subjected to immunoblotting.

2.9. Statistical analysis

All experiments were repeated at least three times. Means ± S.D. were calculated for each group and Dunnet's '*t*' test was used to cal-

culate the statistical significance. A P value <0.05 was considered significant.

3. Results

3.1. Effects of kahweol and cafestol on PGE_2 production in LPS-activated macrophages

In order to investigate their anti-inflammatory effects, kahweol and cafestol (Fig. 1) were tested with regard to their effect on PGE_2 production in the LPS-activated RAW 264.7 macrophages. When the cells were treated with the various concentrations of kahweol and cafestol, the PGE_2 production induced by LPS was significantly inhibited in a dose-dependent manner (Fig. 2), and the kahweol was much more effective in inhibiting PGE_2 production than cafestol. The cell viability was assessed by a MTT assay. An examination of the cytotoxicity of kahweol and cafestol in the RAW 264.7 macrophages indicated that these compounds did not adversely affect the cell viability (>90% cell viability, Fig. 2). Therefore, the inhibition of LPS-induced PGE_2 production by kahweol and cafestol was not the result of their cytotoxicity against the cells.

3.2. Effects of kahweol and cafestol on the COX-2 expression in LPS-activated macrophages

Western blotting of the cell lysate protein was carried out in order to determine if the above effects on PGE_2 production were related to differences in the COX levels. LPS induced COX-2 in the macrophages, and a treatment with kahweol and cafestol caused a dose-dependent decrease in the LPS-mediated induction of COX-2 (Fig. 3A). Kahweol is much more effective at inhibiting COX-2 expression than cafestol. Neither LPS nor kahweol and cafestol affected the COX-1 level (data not shown).

The observed changes in the COX-2 protein level might be a reflection of a change in protein synthesis or degradation. The COX-2 mRNA levels were measured by RT-PCR analysis in order to further elucidate the mechanism responsible for the changes in the amount of the COX-2 protein. Kahweol and cafestol markedly decreased the COX-2 mRNA levels induced by LPS (Fig. 3B). This suggests that kahweol and cafestol suppress COX-2 expression at the transcriptional level, thereby contributing to decreasing the production of the COX-2 protein and PGE_2.

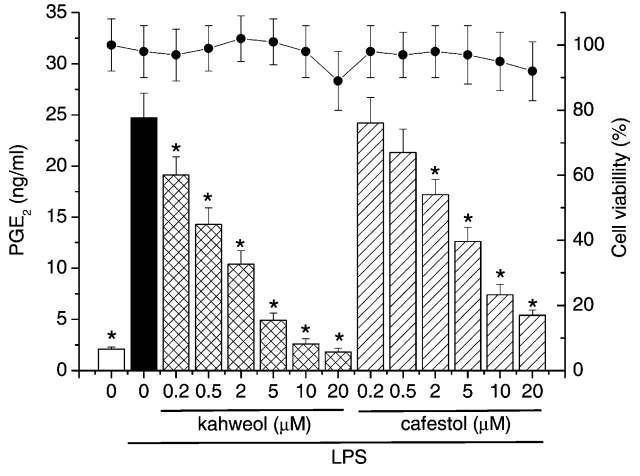

Fig. 2. The effects of kahweol and cafestol on PGE_2 production. The cells were treated with kahweol or cafestol in the presence of LPS (0.5 μg/ml). The supernatants were harvested 24 h later and assayed for PGE_2 production. The cell viability was evaluated with the MTT assay (solid line connecting solid circles). The results are presented as a percentage of the control value obtained from non-treated cells. The values are expressed as means ± S.D. of three individual experiments, performed in triplicate. *$P < 0.01$ compared with the LPS alone.

3.3. Effects of kahweol on the activation of NF-κB in LPS-activated macrophages

NF-κB activation is essential for the induction of COX-2 by LPS or other inflammatory cytokines [5] and kahweol is much more effective at inhibiting PGE_2 production and COX-2 expression by LPS than cafestol. Therefore, this study investigated whether or not kahweol could suppress NF-κB activation in the LPS-activated macrophages using an electrophoretic mobility shift assay. The induction of the NF-κB binding activity by LPS was markedly inhibited by kahweol in a dose-dependent manner (Fig. 4A, upper panel). The addition of an excessive quantity of an unlabeled wild type probe completely prevented the NF-κB binding (Fig. 4A, upper panel), demonstrating the binding specificity of the NF-κB complex. The addition of anti-p65 or anti-p50 antibody to the reaction mixture obtained from the LPS-treated cells caused a supershift in NF-κB binding, whereas the anti-RelB antibody did not shift the retarded band (Fig. 4A, lower panel). Supershift assays using antisera against p50, p65, or RelB indicated that this protein complex contained the p50 and p65 subunits of NF-κB.

In order to further investigate the importance of LPS and kahweol in modulating the expression of COX-2 and NF-κB

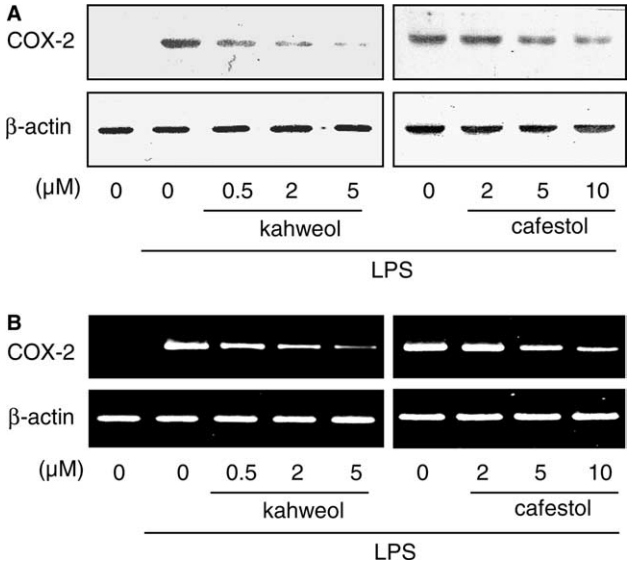

Fig. 3. The effect of kahweol and cafestol on LPS-induced expression of the COX-2 protein and mRNA. The cells were treated with either kahweol or cafestol in the presence of LPS (0.5 μg/ml). (A) Immunoblot analysis. After 24 h of incubation, the cell lysates (30 μg protein) were separated by SDS–PAGE, transferred to a nitrocellulose membrane and blotted with an anti-COX-2 or β-actin antibody. (B) RT-PCR analysis. After 3 h of incubation, the total RNA was prepared and RT-PCR was performed as described in Section 2. The PCR products were separated on a 1.2% agarose gel and stained with ethidium bromide. These blots (A and B) are representatives of each of three independent experiments.

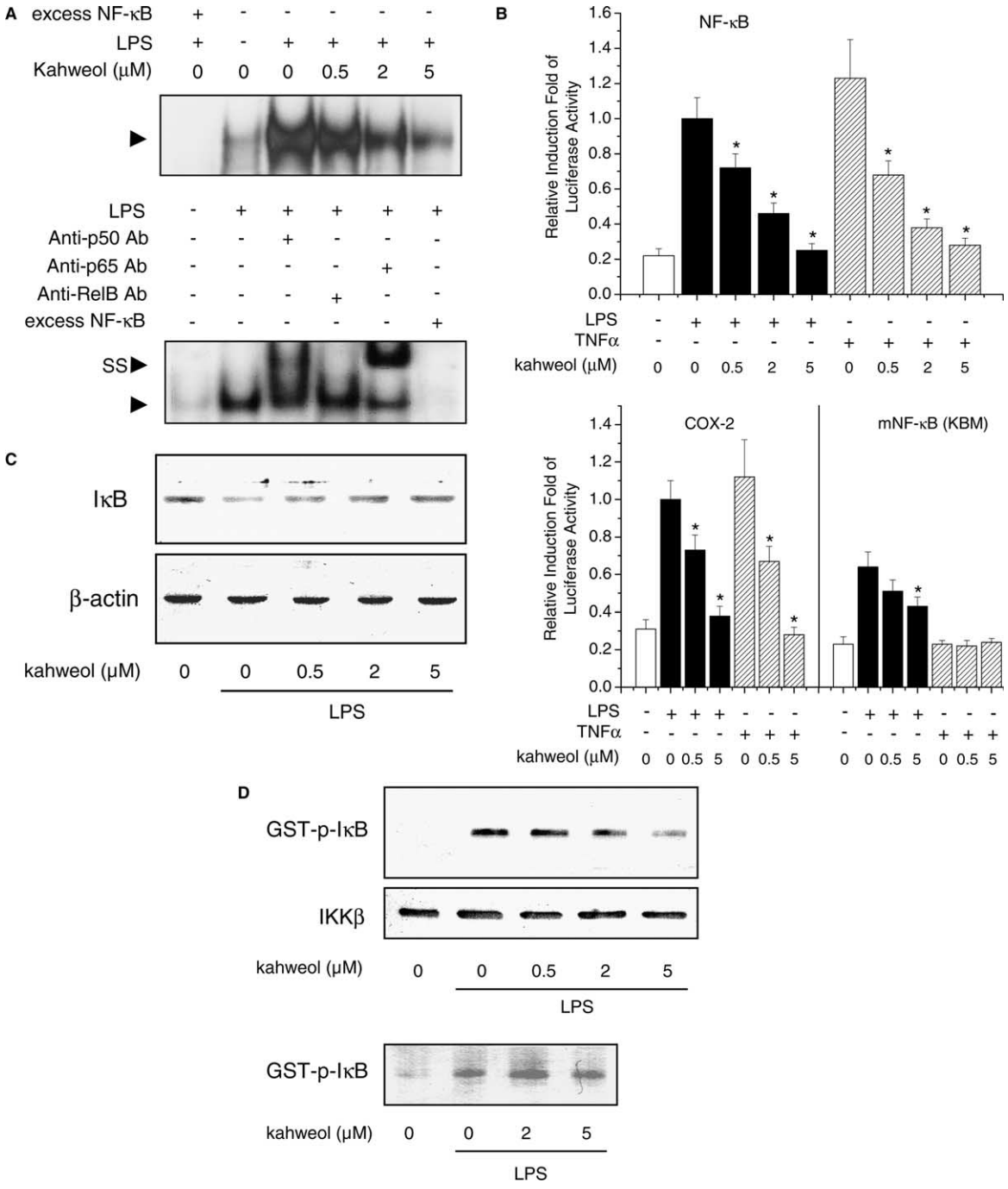

Fig. 4. Effects of kahweol on (A) LPS-induced NF-κB activity determined by EMSA, (B) pNF-B-Luc and COX-2 promoter-Luc reporter plasmids, (C) IκB degradation and (D) LPS-induced IκB kinase activity. (A) The cells were treated with kahweol and LPS (0.5 μg/ml) for 30 min. The nuclear extracts were prepared and EMSA was carried out (upper panel). The specificity of NF-κB binding was confirmed by supershift analysis using the antibodies directed against p65, p50, or RelB protein (lower panel). The arrow indicates the NF-κB binding complex and SS indicates supershift of the retarded NF-κB band. Excess NF-κB; 200-fold molar excess of non-labeled NF-κB probe. (B) The cells were transiently co-transfected with pGL3-4κB-Luc, COX-2 promoter-Luc, or NF-κB mutant COX-2 promoter-Luc (mNF-κB, KBM) and pCMV-β-gal. After 18 h, the cells were treated with kahweol in the presence of LPS (0.5 μg/ml) or TNFα (5 ng/ml) for 12 h, harvested and their luciferase and β-galactosidase activities were determined. The luciferase activities were expressed relative to the LPS. The values are expressed as means ± S.D. of three individual experiments, performed in triplicate. *$P < 0.01$ compared with the LPS or TNFα alone. (C) The cells were treated with kahweol and LPS (0.5 μg/ml) for 30 min. Total cellular protein (50 μg) was separated on 10% SDS–PAGE and blotted with antibody specific for IκB. (D) The cells were treated with kahweol and LPS (0.5 μg/ml) for 30 min and IKK was immunoprecipitated from cell lysates using IKKβ Ab. The activity of immunoprecipitated IKK was measured using GST-IκBα as substrate and GST-p-IκBα was visualized by autoradiography. Relative amount of IKKβ in the precipitated complex was determined by immunoblot (upper panel). IKK was immunoprecipitated from LPS-activated cells and IKK activity was measured in the absence or presence of kahweol added to the assay mixture. IKK activity was assessed using GST-IκBα as described for upper panel (lower panel). These blots (A, C, and D) are representatives of each of three independent experiments.

activity in the LPS-activated macrophages, transient transfections were performed using the COX-2 luciferase promoter construct and the NF-κB-dependent luciferase reporter plasmid. Kahweol inhibited the LPS- or TNFα-activated COX-2 promoter activity and NF-κB transcriptional activity (Fig. 4B). These results suggest that the suppression of COX-2 expression by kahweol occurred via the inhibition of NF-κB activation. In addition, to evaluate the role of the NF-κB site in the expression of COX-2 gene and to determine the effects of kahweol on the NF-κB activation by LPS, the cells were transfected with the luciferase reporter vector KBM which is driven by the COX-2 promoter region (−327/+59) specifically mutated at its NF-κB site (−223/−214) [17]. When the cells were transfected with KBM, although LPS-inducible promoter activity was decreased, kahweol further inhibited LPS-activated promoter activity (Fig. 4B). These results suggest that the other *cis*-acting element(s) may also be responsible for the effects of kahweol on COX-2 expression. The experiments to assess whether kahweol changes the activities of other transcriptions factors (i.e., NF-IL-6, CRE) required for the COX-2 gene expression remain to be carried out. In contrast, mutation of the NF-κB site abolished the enhanced promoter activity by TNFα (Fig. 4B). NF-κB site at −223/−214 is critical for TNFα-induced COX-2 expression consistent with reported results [19]. Taken together, these results suggest that the NFκB is involved in the LPS-induced COX-2 expression and the suppression of COX-2 expression by kahweol occurred via the inhibition of NF-κB activation.

Since it has been well documented that NF-κB activation correlates with the rapid proteolytic degradation of IκB, the prevention of IκB degradation was also examined as an indication that kahweol inhibits NF-κB activation. LPS induced a transient degradation of IκBα in the cells, whereas kahweol prevented the degradation of IκB (Fig. 4C). Since IκB is phosphorylated by the IKK, the effect of kahweol on the cellular IKK activation was determined (Fig. 4D). The cells were activated by LPS in the presence of kahweol. The IKK complex was immunoprecipitated from cell lysates and analyzed for IKK activity using GST-IκBα as substrate. The blot used for the autoradiogram was subsequently probed for IKKβ by immunoblot analysis. Kahweol significantly inhibited the IKK activity induced by LPS (Fig. 4D). However, kahweol had little or no effect on the IKK protein level (Fig. 4D, upper panel), suggesting that the inhibition of LPS-induced IKK activity by kahweol was not due to the decreased IKK expression level. Additional experiments were carried out to more stringently test the effect of kahweol on IKK activity. IKKβ was immunoprecipitated from the cell lysates of the LPS-activated cells and kahweol was added at the beginning of the assay for IKK activity. The activity of IKK was not affected by the presence of kahweol (Fig. 4D, lower panel). These results suggest that the inhibition of COX-2 expression by kahweol occurred via the suppression of IKK activity, which resulted in the prevention of NF-κB activation.

4. Discussion

Recent studies have shown that kahweol and cafestol have anti-carcinogenic effects. The results in this study link the effects of kahweol and cafestol to the inhibition of PGE_2 production. With regard to PGE_2 production, it was shown that kahweol and cafestol inhibit the LPS-activated induction of COX-2 in the macrophages.

The chemopreventive or anti-carcinogenic properties of kahweol and cafestol can be understood, at least in part, from the induction of several beneficial modifications to the xenobiotic metabolism, which, depending on the individual compound, may involve both the reduced activation and enhanced detoxification of mutagens/carcinogens [11–13,15]. Epidemiological studies have revealed a protective association between coffee consumption and the risk of certain types of cancer including colon cancer [14,20,21]. Meanwhile, COX-2 has been implicated in the carcinogenic processes [2], and its over-expression by malignant cells has been shown to enhance cellular invasion, induce angiogenesis, regulate the anti-apoptotic cellular defenses and augment the immunological resistance via PGE_2 production [22]. In addition, it has been demonstrated that COX-2 is over-expressed in colon cancer patients [23].

There is growing evidence to suggest that inhibitors of COX-2 activity can be effective anti-inflammatory agents, as well as being beneficial in the prevention and treatment of colon cancer [3,24]. Therefore, agents that interfere with the signaling mechanisms governing the transcription of COX-2 should also inhibit inflammation and tumorigenesis [3,4]. The major focus of this study was to investigate the effects of kahweol and cafestol on COX-2 expression using a macrophage model. This study showed that kahweol and cafestol, which are both coffee diterpenes, inhibit PGE_2 production in LPS-stimulated macrophages in a dose-dependent manner. Moreover, kahweol had a larger effect on PGE_2 production than cafestol (Fig. 2). This suggests the possible suppression of COX-2 induction by these compounds. Therefore, the effect of kahweol and cafestol on COX-2 expression was investigated in order to obtain a better understanding of the inhibitory mechanism of PGE_2 production. It was found that the simultaneous treatment of either kahweol or cafestol with LPS significantly inhibited COX-2 mRNA and protein expression (Fig. 3). Kahweol had a more potent inhibitory effect on PGE_2 production and the COX-2 expression than cafestol. These phenomena might depend on the structures of these two diterpenes (Fig. 1). The absence of one double bond on the A ring of kahweol increases its potency in suppressing COX-2 expression in a different manner to that of cafestol. The presence of this single double bond within the A ring of cafestol appears to be less effective in inhibiting COX-2 expression than kahweol. Future experiments will be needed to determine the relationship between the structures of these two diterpenes and their different efficacy. The administration of kahweol and cafestol (2–10 mg/kg, i.p.) was also observed to block COX-2 expression in the cells present in the exudate and produced a dose-dependent decrease in PGE_2 production in an in vivo mouse air pouch model of carrageenan-induced inflammation (data not shown).

Animal studies have demonstrated that kahweol and cafestol have chemoprotective properties (anti-carcinogenic effects) against carcinogens [11–13]. Carcinogenesis typically involves a cellular transformation, hyperproliferation, invasion, angiogenesis, and metastasis. Various carcinogens, inflammatory agents, and tumor promoters activate these processes. Carcinogenic agents, such as nitrosamines, 7,12-dimethylbenz[*a*]anthracene, and aflatoxin B_1 have been shown to activate NF-κB [25,26]. These agents have been used to examine the anti-carcinogenic effects of kahweol and cafestol in animal studies.

Furthermore, NF-κB has been shown to regulate the expression of a number of genes whose products are involved in carcinogenesis/tumorigenesis [27,28]. Because the suppression of NF-κB has been implicated in chemoprevention, it is also possible that the anti-carcinogenic effects of kahweol are mediated via the suppression of NF-κB-dependent gene expression.

This study demonstrated that kahweol and cafestol suppress the LPS-activated expression of COX-2, which has NF-κB binding sites in its promoter, and regulate its transcription in macrophages. Furthermore, it was found that kahweol inhibited the LPS-induced activation of IKK, IκBα phosphorylation and degradation. The phosphorylation of IκB is regulated by IKK, which in turn is regulated by many upstream kinases, including NIK, Akt, and mitogen-activated protein kinase kinase kinase 1 [6,7,27]. This study found that kahweol did not directly affect the activity of IKK (Fig. 4D), which suggests that kahweol inhibits the LPS-induced IKK activity by an indirect mechanism. Therefore, it is possible that kahweol inhibits IKK activation by inhibiting one or more of the upstream kinases responsible for IKK activation.

Although the down-regulatory ability of kahweol on COX-2 expression was demonstrated by the inhibition of NF-κB activation in the LPS-stimulated macrophages, the precise mechanism by which kahweol suppresses COX-2 expression in the macrophages and exerts its anti-inflammatory effects is still largely unknown. The activation of the MAPK members, ERK and p38 MAPK, have been shown to be involved in the stimulation of NF-κB activity and the subsequent expression of COX-2 in the LPS-activated macrophages [29]. Kahweol might also inhibit the activity of these kinases, leading to NF-κB activation before or during the IκB phosphorylation step. The suppression of NF-κB activation by kahweol may partially account for this. This is because there are responsive elements on the promoters of the COX-2 gene. However, other *cis*-acting elements such as the CRE and the NF-IL6 site are also involved in the transcriptional regulation of the COX-2 gene [17]. Therefore, further studies of the effects of kahweol on the other *cis*-acting elements are necessary to understand the regulation of the COX-2 gene expression by kahweol and clarify the mechanisms involved.

In conclusion, the coffee-specific diterpenes, kahweol and cafestol, were found to inhibit PGE_2 production and COX-2 expression in macrophages via the inhibition of NF-κB activation. These novel findings may help identify other mechanisms for the anti-inflammatory or cancer chemopreventive activities, and provide new insights into the previously unrecognized biological activity of kahweol and cafestol.

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