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Luteolin inhibits the nuclear factor-κB transcriptional activity in Rat-1 fibroblasts

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Abstract

Flavonoids are natural polyphenolic compounds that have anti-inflammatory, cytoprotective and anticarcinogenic effects. In this study, we investigated the effects of several flavonoids on nuclear factor-kappa B (NF-κB) activation by using luciferase reporter gene assay. Among the flavonoids examined, luteolin showed the most potent inhibition on lipopolysaccharide (LPS)-stimulated NF-κB transcriptional activity in Rat-1 fibroblasts. Luteolin did not inhibit either IκBα degradation or NF-κB nuclear translocation, DNA binding or phosphorylation by LPS. However, luteolin prevented LPS-stimulated interaction between the p65 subunit of NF-κB and the transcriptional coactivator CBP. In addition, a specific PKA inhibitor that blocked the phosphorylation of CREB and c-Jun by luteolin partially reversed the inhibitory effect of luteolin on NF-κB·CBP complex formation and NF-κB transcriptional activity by LPS. These data imply that inhibition of NF-κB transcriptional activity by luteolin may occur through competition with transcription factors for coactivator that is available in limited amounts. Taken together, this study provides a molecular basis for the understanding of the anti-inflammatory effects of luteolin.

Keywords: Nuclear factor-kappa B; Luteolin; Coactivator; Lipopolysaccharide; Fibroblast; Anti-inflammation

1. Introduction

Flavonoids are a group of about 4000 naturally occurring polyphenolic compounds that are ubiquitous in all vascular plants. They are classified into several subgroups including flavonols, flavones, isoflavones, and flavanones according to the presence of different substituents on the benzene rings and the degree of benzo-γ-pirone ring saturation. Flavonoids without a sugar molecule are able to pass through the cell membrane. Flavonoids, once absorbed, influence many biological functions including protein synthesis, cell proliferation and angiogenesis [1]. Luteolin is a 3′,4′,5,7-tetrahydroxyflavone and usually occurs as glycosylated forms in celery, green pepper, perilla leaf and camomile tea [2]. It has been found to possess antimutagenic [3], antitumorigenic [4], antioxidant [5] and anti-inflammatory properties [6]. Several reports have suggested that the anti-inflammatory effects of luteolin are mediated through the inhibition of nitric oxide release [5], cytokine production, protein tyrosine phosphorylation and NF-κB-mediated gene expression [6]. However, the molecular mechanism is still unclear.

Nuclear factor-κB is a ubiquitous transcription factor and has a highly Rel homology domain, which controls DNA binding, dimerization and interactions with inhibitory proteins, IκB. In unstimulated cells, the NF-κB complex, which associates with IκB, is localized in the cytoplasm. Upon stimulation by tumor necrosis factor (TNF), interleukin-1 (IL-1) or bacterial LPS, IκB is phosphorylated, ubiquitinated and degraded. The removal of IκB allows NF-κB translocation to the nucleus where it binds to

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Abbreviations: NF-κB, nuclear factor-kappa B; IκB, inhibitor of NF-κB; CREB, cyclic AMP response element-binding protein; AP-1, activator protein-1; CBP, CREB-binding protein; LPS, lipopolysaccharide.

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specific κB elements within the promoter regions of genes involved in immune response and inflammation [7].

Several studies have shown that the interactions between NF-κB and coactivators are essential for NF-κB transcriptional activity [8,9]. Coactivators link transcription factors to the basal transcriptional machineries such as the TATA box-binding protein (TBP), TFIIB and histone acetyltransferases [9]. Since they are present in limited amounts in the nucleus and are capable of interacting with several transcription factors, including CREB [10], c-Fos [11], c-Jun [12] and p53 [13], competitions between transcription factors for coactivators provide another mechanism for transcriptional regulation [14–16].

In this study, we examined the effects of falvonoids on LPS-induced NF-κB transcriptional activity in Rat-1 fibroblasts. Of the flavonoids examined, luteolin showed the most potent inhibition on LPS-induced NF-κB transcriptional activity through the reduction of the interaction between NF-κB and CBP. Our results suggest a novel molecular mechanism for the regulation of NF-κB-dependent transcription by luteolin.

2. Materials and methods

2.1. Materials

DMEM, G418 and LipofectAmine were purchased from Gibco BRL. Luteolin, apigenin, genistein, formonetin, quercetin, morin, narigerin and LPS (form *Escherchia coli* 055: B5) were obtained from Sigma–Aldrich. Antibodies against p65, IκBα and c-Jun were purchased from Santa Cruz Biotechnology, and antibodies against phosphorylated-CREB and phosphorylated-c-Jun were obtained from New England Biolabs. Biotinylated secondary antibody and fluorescein streptavidin were purchased from VECTOR Laboratories. (κB)4-luciferase plasmid was obtained from Stratagene. Oligonucleotides and luciferase activity assay reagent were purchased from Promega. PKA inhibitor, H89, was obtained from Calbiochem.

2.2. Cell culture

Rat-1 fibroblasts were maintained in DMEM supplemented with 10% fetal bovine serum. Before stimulation, cells were starved with medium containing 0.1% serum for 2 days.

2.3. Stable transfection

Rat-1 cells were transfected with plasmid DNA using LipofectAmine reagent according to a procedure recommended by the manufacturer. To establish a stable cell line, cells were cotransfected with pNF-κB-Luc plasmid and pcDNA 3.1(+) including neomycin-resistant gene. Positive clones were selected in the culture media containing 700 μg/mL G418 and their transcriptional responses were tested by luciferase activity assay after treatment with 1 μg/mL LPS.

2.4. Luciferase assay

Cells were lyzed with lysis buffer (20 mM Tris–HCl, pH 7.8, 1% Triton X-100, 150 mM NaCl, 2 mM DTT). The cell lysate 5 μL was mixed with luciferase activity assay reagent 25 μL and luminescence produced for 5 s was measured using luminoskan (labsystems).

2.5. Immunoprecipitation and Western blotting

Whole cell extracts were prepared using IP buffer (10 mM Tris–HCl, pH 7.8, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 1 μg/mL lepeptin, 1 μg/mL aprotinin) according to previously described procedure [17]. Antibody against CBP was incubated with 20 μL of protein A beads for 2 hr at 4°. Complexes of proteins with CBP were precipitated by combining extracts with the beads and incubation for 4 hr at 4°. Beads were centrifuged and pellets washed three times with lysis buffer prior to addition of SDS loading buffer. For Western blot analysis, whole cell lysates were prepared in lysis buffer (20 mM Tris–HCl, pH 8.0, 150 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF). Electrophoresis was performed with 8% polyacrylamide gels and then the proteins were electrotransferred to nitrocellulose membranes. Membranes were immunoblotted with polyclonal antibodies against IκBα, phosphorylated-CREB or phosphorylated-c-Jun, or with monoclonal antibody against p65 in Tween-20/Tris-buffered saline containing 5% skimmed milk. After incubation with an appropriate peroxidase-conjugated secondary antibody, proteins were detected with the enhanced chemiluminescence system (ECL system from Amersham).

2.6. MTT assay

To determine cell viability, 1×10^4 cells/well were subcultured in a 96-well plate. After treatment with luteolin in serum-free DMEM for the indicated times, the cells were incubated for 1 hr in the presence of 0.5 mg/mL MTT reagent in a 37° incubator. After removal of MTT reagent and disruption of cells with DMSO, the absorbance was measured at 570 nm using a 96-well plate reader. Data are presented as the percentage of viability relative to vehicle-treated control cultures.

2.7. Immunocytochemistry

Cells were washed with cold PBS twice, fixed with 4% paraformaldehyde in PBS for 30 min and cold MeOH for 10 min, and then permeabilized with sodium citrate/0.1% TX-100 in PBS for 5 min at 4°. After incubating cells with

primary antibody (monoclonal anti-p65 antibody, 1:100) in 1% BSA/0.1% TX-100 in PBS for 90 min, they were sequentially incubated with biotinylated secondary antibody for 40 min, and then fluorescein streptavidin for 30 min. Sequentially, p65 was examined under a fluorescence microscope (Carl Zeiss).

2.8. Nuclear extract preparation

Nuclear extracts were prepared by the procedure of Suk *et al.* [18]. Cells were treated with LPS for 1 hr after pretreatment with luteolin for 1 hr. And then washed with ice-cold PBS twice, harvested, and pelleted by centrifugation at 1500 *g* for 5 min. After resuspending in 1 mL of Solution A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 1 μg/mL lepeptin, 1 μg/mL aprotinin) and incubating for 20 min with rotation at 4°, the samples were centrifuged at 14,000 *g* for 20 s and the supernatant was removed. The nuclear pellets were resuspended in 30 μL of Solution B (20 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 420 mM NaCl, 0.2 mM EDTA, 0.5 mM DTT, 25% glycerol, 0.5 mM PMSF, 1 μg/mL leupeptin, 1 μg/mL aprotinin), incubated on the ice for 30 min, and 30 μL of Solution C (20 mM HEPES, pH 7.9, 0.2 mM EDTA, 0.5 mM DTT, 25% glycerol, 0.5 mM PMSF, 1 μg/mL leupeptin, 1 μg/mL aprotinin) were added. The samples were centrifuged at 14,000 *g* for 15 min at 4°. The nuclear extract was collected and frozen at −80°.

2.9. Electrophoretic mobility shift assay

EMSA was performed as described previously [18]. Oligonucleotides containing the consensus binding sequence for NF-κB (5′-agttgaggggactttcccaggc-3′) were end-labeled with T4 polynucleotide kinase (Promega) using 30 μCi of γ-^{32}P-labeled ATP (NEN). For each sample, 10 μg of nuclear extracts were incubated with the labeled oligonucleotides for 10 min at RT in band shift buffer (10 mM HEPES, pH 7.9, 60 mM NaCl, 1 mM EDTA, pH 8.0, 7% glycerol) containing 1 μg/mL of poly(dI-dC) (Sigma) as a nonspecific competitor. Electrophoresis was carried out using 6% nondenaturing polyacrylamide gel (6% acrylamide, 0.25× Tris borate-EDTA (TBE), 2.5% glycerol), gels were dried and followed by autoradiography. Nuclear extracts were preincubated with antibody for shift of oligonucleotides and NF-κB complex.

2.10. In vivo phosphorylation of p65

In vivo Rat-1 cells were labeled with 1 mCi of ^{32}P (NEN) in phosphate-free DMEM medium for 3 hr at 37° and then pretreated for 1 hr with luteolin prior to the addition of LPS for 1 hr. After washing with PBS, the cells were suspended in RIPA buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 1% deoxycholate, 1% NP-40, 0.1% SDS, 5% glycerol, 1 mM EGTA, 10 mM K_2HPO_4, 50 mM NaF, 1 mM PMSF, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 30 mM sodium pyrophosphate, 30 mM glycerophosphate), and sonicated. The p65 was immunoprecipitated with mouse anti-p65 antibody coupled to the protein A-agarose beads (Sigma). The precipitated proteins were washed several times with ice-cold RIPA buffer, followed by electrophoresis on 8% SDS–PAGE gel and autoradiograpy.

3. Results

3.1. Luteolin inhibits LPS-induced NF-κB transcriptional activity in Rat-1 fibroblasts

To investigate the anti-inflammatory effect of flavonoids, nine flavonoids were examined with regard to their effect on LPS-induced NF-κB transcriptional activity in Rat-1 fibroblasts. Rat-1 cells were stably transfected with the (κB)4-luciferase reporter plasmid, containing four copies of the NF-κB consensus site. In the transfected cells, LPS increased NF-κB-mediated transcriptional activity in a dose-dependent manner (Fig. 1A). Cells were pretreated with 20 μM of each flavonoid for 1 hr and subsequently treated with 1 μg/mL LPS for 6 hr. Among the flavonoids examined, luteolin showed the most potent inhibition for NF-κB transcriptional activity (Fig. 1B). As shown in Fig. 2A, luteolin alone had no effect on basal NF-κB-dependent transcription, whereas LPS-increased NF-κB transcriptional activity was dose-dependently reduced by luteolin. Since it was reported that high concentration of luteolin reduced the viability of RAW 264.7 cells [5], we examined the cytotoxic effect of luteolin in Rat-1 cells by MTT assay. When cells were treated with the indicated concentrations of luteolin for 12 hr, they showed no change in the viability up to 20 μM (Fig. 2B).

3.2. Luteolin does not inhibit LPS-induced IκBα degradation

The translocation of NF-κB into the nucleus is linked to proteolytic degradation of IκBα [7]. To determine whether luteolin interferes with LPS-induced IκBα degradation, the level of IκBα protein was examined by Western blotting. As expected, we observed a time-dependent IκBα degradation in LPS-stimulated cells (Fig. 3A). However, luteolin did not affect on LPS-induced IκBα degradation, as shown in Fig. 3A.

3.3. Luteolin has no effects on LPS-induced NF-κB nuclear translocation, DNA binding and phosphorylation

Since luteolin inhibited LPS-induced NF-κB transcriptional activity without a diminution of IκBα degradation, we examined the effect of luteolin on the translocation

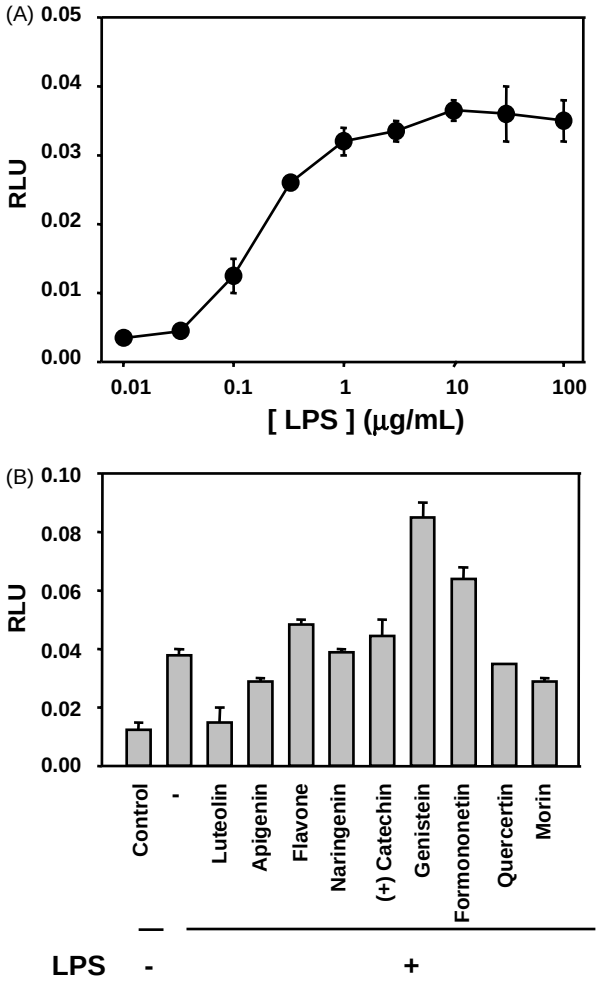

Fig. 1. Effects of flavonoids on LPS-induced NF-κB transcriptional activity. Cells were stably transfected with (κB)4-luciferase construct as described in Section 2. (A) Cells were treated with LPS at the indicated concentrations for 6 hr. (B) Cells were pretreated with 20 μM flavonoids for 1 hr and subsequently treated with 1 μg/mL LPS for an additional 6 hr. Cells were lyzed for luciferase assay. Data present the means ± SD of three experiments. RLU: relative light unit.

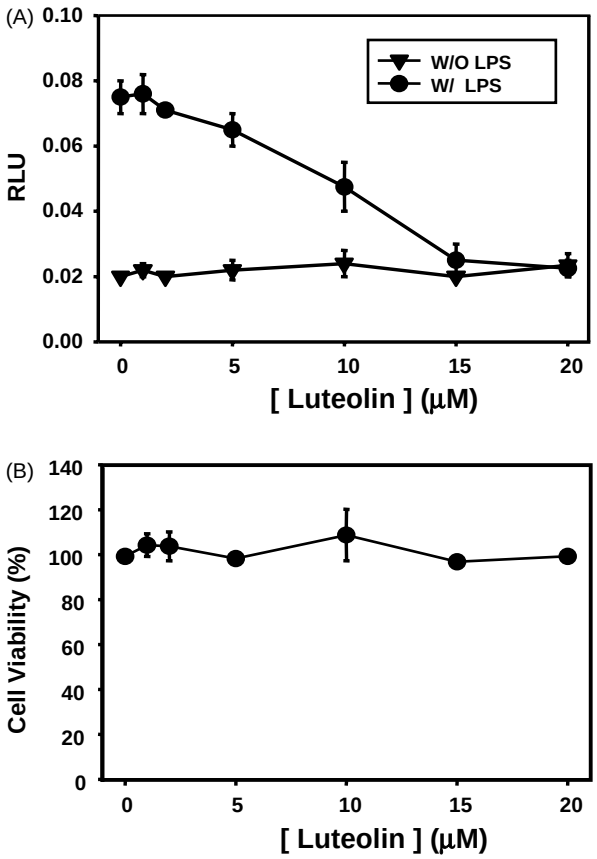

Fig. 2. Effects of luteolin on LPS-induced NF-κB transcriptional activity. (A) After pretreated with luteolin at the indicated concentrations for 1 hr and then treated with LPS for 6 hr, the transfected cells were lyzed to determine the expression of luciferase. (B) After the transfected cells were treated with the indicated concentrations of luteolin for 24 hr, cell viability was determined by MTT assay performed as described in Section 2.

of NF-κB into the nucleus by immunocytochemistry. Stimulation of Rat-1 cells with LPS for 1 hr led to strong NF-κB nuclear translocation, and this was similar in the absence or the presence of luteolin (Fig. 3B). To assess whether luteolin inhibits NF-κB-dependent transcription by inhibiting the binding of NF-κB to DNA, EMSA was performed. As shown in Fig. 3C, incubation with LPS for 1 hr increased NF-κB DNA binding activity and its specific binding was confirmed with antibody against p65 that induced supershift. Induction of NF-κB DNA binding activity by LPS was not changed by luteolin (Fig. 3C). These results indicate that luteolin does not inhibit NF-κB transcriptional activity at the level of NF-κB nuclear translocation or DNA binding.

Several studies have demonstrated that p65 is phosphorylated during activation, which lead to its increased transcriptional activity [19]; moreover, hindrance of p65 phosphorylation inhibits NF-κB transcriptional activity [20]. Therefore, we examined the effect of luteolin on LPS-induced p65 phosphorylation. LPS was found to induce p65 phosphorylation and luteolin to cause a slight induction. Moreover, luteolin somewhat potentiated LPS-induced p65 phosphorylation (Fig. 3D, upper panel). Similar levels of p65 protein were detected in the absence or presence of LPS or luteolin (Fig. 3D, lower panel).

3.4. Luteolin inhibits the interaction of NF-κB with CBP through increasing phosphorylation of CREB and c-Jun

Transcriptional competence of NF-κB requires the interaction with CBP [8,9]. To examine the effects of luteolin on this interaction, cells were stimulated with LPS in the absence or presence of luteolin, and total cell lysates were immunoprecipitated with antibody against CBP and probed for the presence of NF-κB. LPS stimulation resulted in the appearance of p65·CBP complex. Luteolin significantly decreased the level of p65·CBP complex formation by LPS (Fig. 4A).

Several reports have stated that the decrease in the interaction of NF-κB with coactivators occurs because

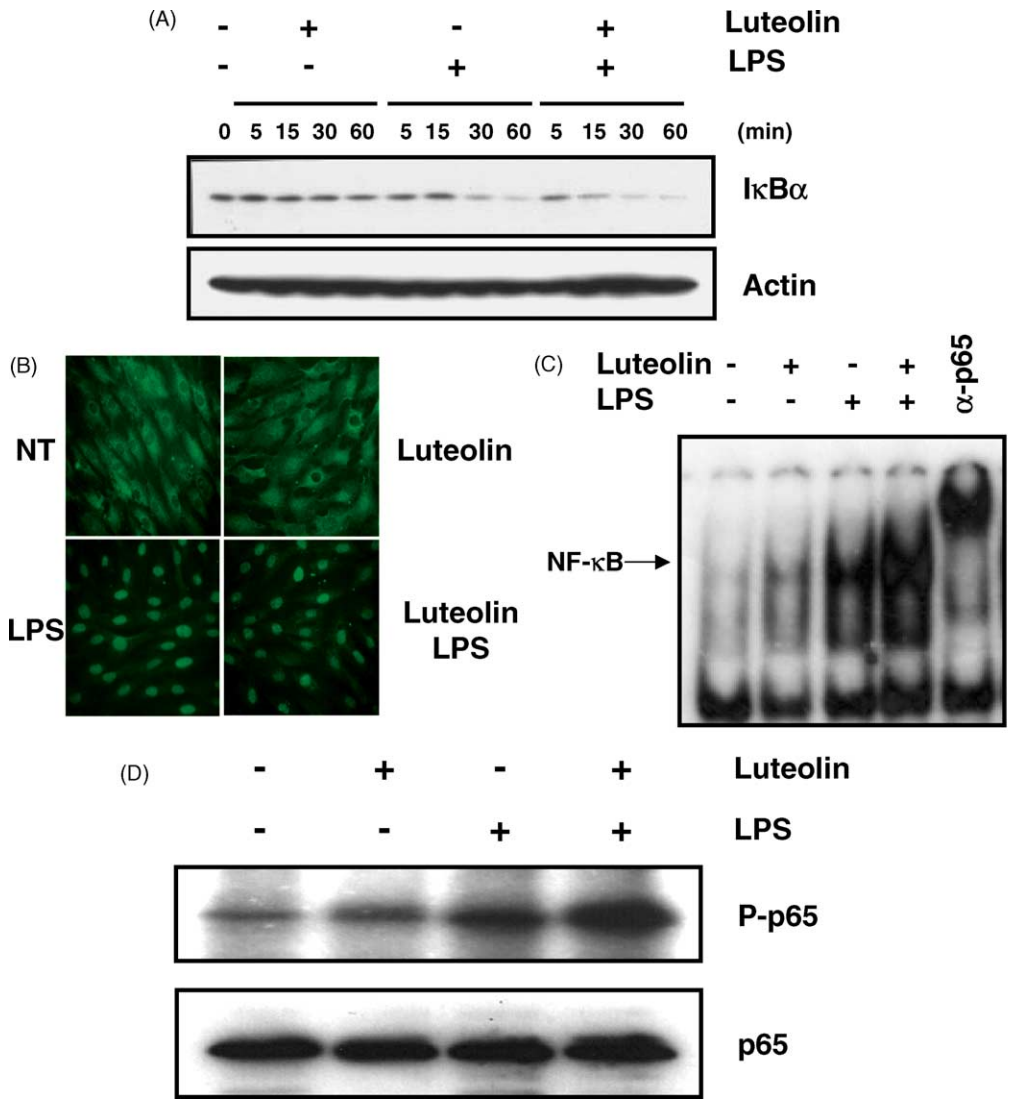

Fig. 3. Effects of luteolin on LPS-induced IκBα degradation, or the nuclear translocation, DNA binding or phosphorylation of NF-κB. (A) Cells were pretreated with 20 μM luteolin for 1 hr, and then stimulated with 1 μg/mL LPS for the indicated times. 15 μg of each protein was separated by SDS–PAGE, and then Western blot analysis with specific antibodies against IκBα or actin was performed. (B) Cells were pretreated with luteolin, and then stimulated with 1 μg/mL LPS for 1 hr. The localization of NF-κB was detected by immunofluorescence with specific antibody against p65 as described in Section 2. (C) Cells were pretreated with luteolin for 1 hr, and the stimulated by the addition of 1 μg/mL LPS for 1 hr. 10 μg of each nuclear protein were subjected to a DNA binding reaction with ^{32}P-end-labeled NF-κB consensus sequence, and then DNA–protein complexes were separated by nave polyacrylamide gel electrophoresis. To check the specific binding of NF-κB, nuclear extracts were preincubated with antibody against p65 for shift of oligonucleotides and NF-κB complex. (D) Phosphate-labeled cells were pretreated with 20 μM luteolin for 1 hr, and then stimulated with the addition of 1 μg/mL LPS. After incubation for 1 hr, p65 was recovered by immunoprecipitation using specific antibody and fractionated by SDS–PAGE, followed by autoradiography. The total amounts of p65 were determined by Western blot analysis.

of competition between transcription factors for coactivators that are available in limited amounts [14,15]. Phosphorylated p65 associates with the CBP, which can bind with phosphorylated CREB and c-Jun [21–23]. Therefore, we investigated whether luteolin induces the phosphorylation of CREB or c-Jun. LPS increased phosphorylation of CREB and c-Jun slightly as compared with unstimulated control. In contrast, luteolin augmented the levels of phosphorylated CREB and c-Jun by LPS (Fig. 4B and C, upper panels). Total levels of CREB and c-Jun were similar in the absence or presence of luteolin or LPS (Fig. 4B and C, lower panels). A specific protein kinase A inhibitor, H89, inhibited the phosphorylation of CREB and c-Jun by luteolin (Fig. 4B and C). Moreover, H89 partially reversed the inhibitory effects of luteolin on LPS-mediated interaction of NF-κB with CBP and NF-κB transcriptional activation (Fig. 4A and D). These results suggest that luteolin increases the phosphorylation of CREB and c-Jun, which then competes with NF-κB for limited amounts of CBP, resulting in decreased p65·CBP complex formation. On the other hand, luteolin did not induce the phosphorylation of CREB and c-Jun in RAW 264.7 cells, which indicates that the molecular mechanism of luteolin is cell-type specific (Figs. 4B, C and 5).

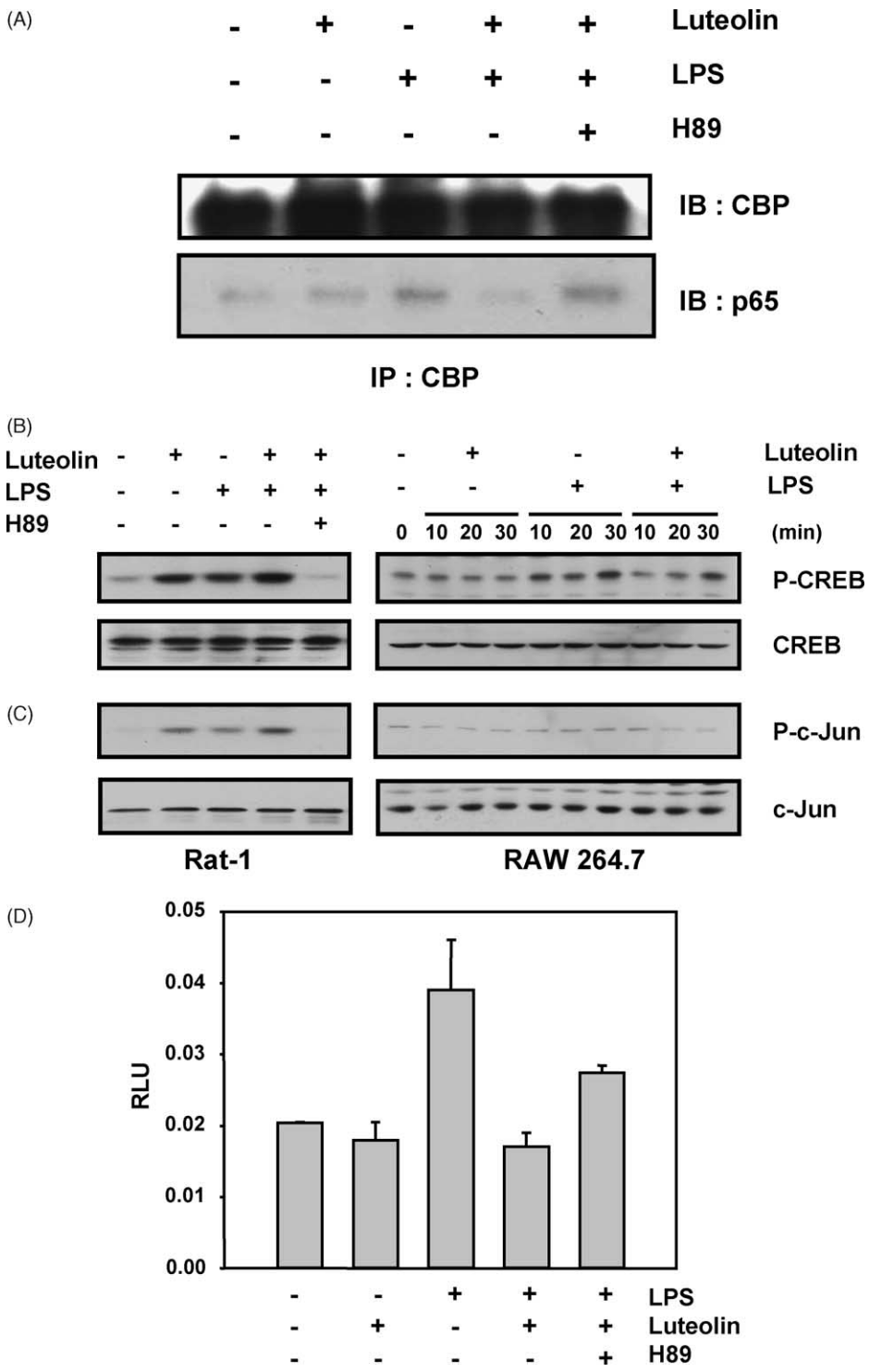

Fig. 4. Effects of luteolin on LPS-induced interaction of NF-κB with CBP. (A) Rat-1 cells were pretreated with 5 μM H89 for 30 min, treated with 20 μM luteolin and 1 μg/mL LPS for 1 hr. Cell extracts were subjected to immunoprecipitation (IP) with anti-CBP and analyzed by Western blot (IB) with anti-NF-κB. (B, C) Rat-1 cells were pretreated with 5 μM H89 for 30 min, treated with 20 μM luteolin and 1 μg/mL LPS for 20 min. RAW 264.7 cells were treated for the indicated times. (B) Using antibody against phospho-CREB revealed rapid phosphorylation of CREB and using antibody against CREB showed no change in levels of CREB protein. (C) Western blotting using antibody against phospho-c-Jun revealed rapid phosphorylation of c-Jun and using antibody against c-Jun showed no change in levels of c-Jun protein. (D) The transfected cells were pretreated with 5 μM H89 for 30 min, treated with 20 μM luteolin, and stimulated with 1 μg/mL LPS. Luciferase activity in cell lysate was assayed. Data present the means ± SD of three experiments performed in duplicate.

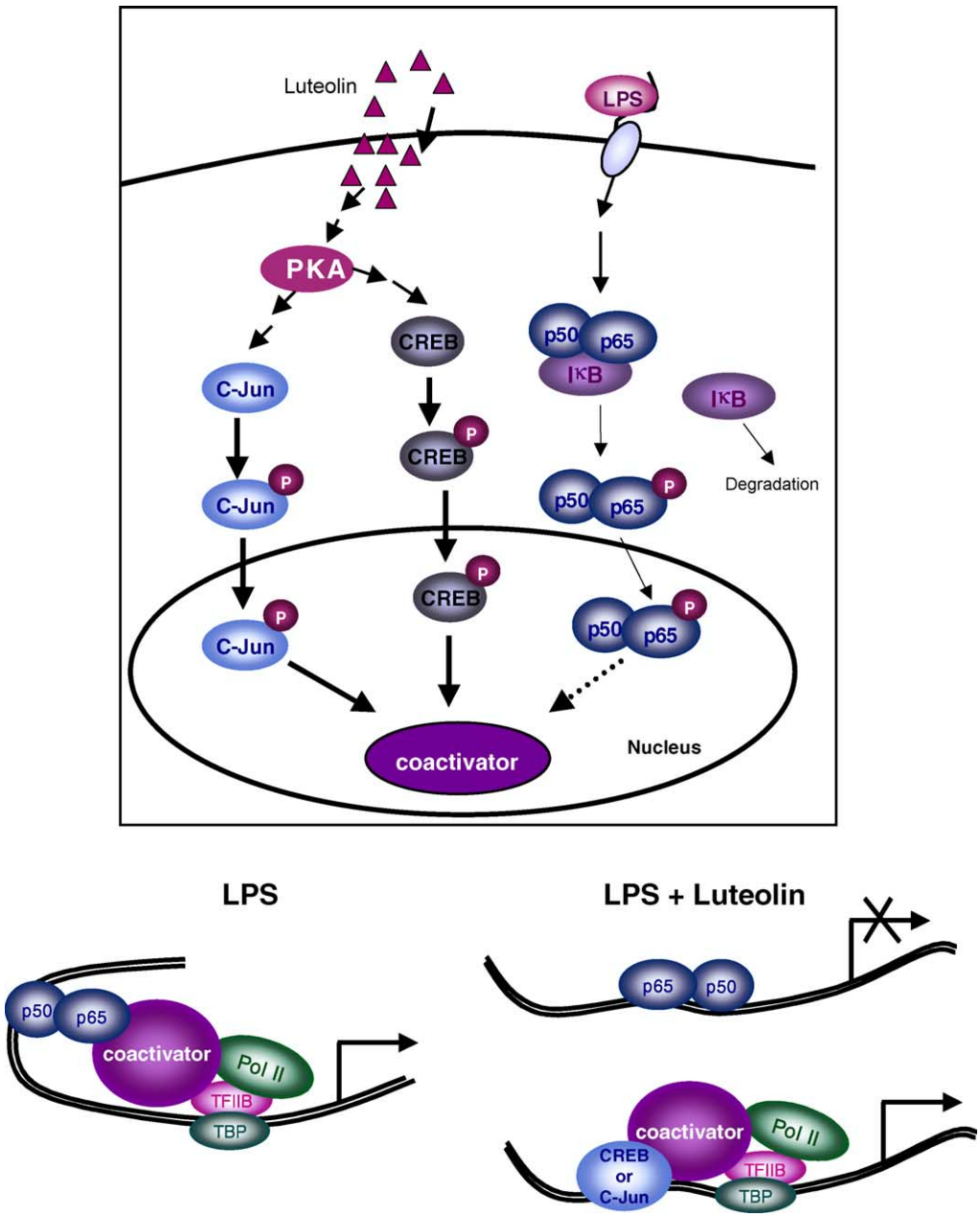

Fig. 5. Model for the inhibitory effect of luteolin on LPS-induced NF-κB-dependent transcriptional activity (see Section 4 for details).

4. Discussion

Flavonoids, a group of polyphenolic natural products, are ubiquitous in the plant kingdom and are found in many traditional herbal medicines. Several flavonoids are biochemically active compounds with anti-inflammatory, anticarcinogenic and free radical scavenging properties. The anti-inflammatory effects of flavonoids were mediated by inhibiting the production of inflammatory mediators such as prostaglandins, leukotrienes, cytokines or nitric oxide [1]. However, the anti-inflammatory effects of some flavonoids are not well established and are different depending on cell-type. Fibroblasts together with macrophages are important sentinel cells in the immune system. Fibroblast activation leads to the rapid production of cytokines, chemokines and prostanoids. However, as fibroblasts are relatively long-lived cells, a control mechanism must exist to prevent over-stimulation of the immune system, which would lead to chronic inflammatory reaction [24]. Recent findings have suggested that the NF-κB signaling pathway appears to play a critical role in perpetuating the inflammatory response at the sites of chronic inflammation [25]. In addition, RelB, one of NF-κB family, can act as a transcriptional activator in fibroblasts but as a transcriptional repressor in hematopoietic cells [24], implying that there are different mechanisms for the regulation of NF-κB signaling pathway in fibroblasts and macrophages. Therefore, it is important to find a mechanism for the regulation

of NF-κB signaling pathway in fibroblasts. Among the flavonoids investigated, luteolin the most potently inhibited NF-κB-dependent transcription by LPS in Rat-1 fibroblasts (Fig. 1B). The inhibitory effects of genistein, apigenin [26] and quercetin [27] on NF-κB signaling have been reported in macrophages; however, quercetin and apigenin did not significantly inhibit NF-κB transcriptional activation in Rat-1 fibroblasts. On the other hand, genistein increased LPS-stimulated NF-κB transcriptional activity in these cells. These results suggest that some flavonoids have different effects depending on cell-type.

We found that luteolin inhibited LPS-induced NF-κB transcriptional activity without prevention of IκBα degradation in Rat-1 fibroblasts (Fig. 3A). In addition, luteolin did not affect NF-κB nuclear translocation, DNA binding and phosphorylation in Rat-1 cells (Fig. 3B, C and D). However, Xagorari *et al.* [6] reported that luteolin inhibited NF-κB-mediated promoter activity by inhibiting IκBα degradation in LPS-treated macrophage RAW 264.7 cells. Moreover, this paper showed that treatment of RAW 264.7 cells with luteolin inhibited LPS-stimulated tyrosine phosphorylation and phosphorylation of AKT. However, in Rat-1 cells luteolin had no inhibitory effects on them (data not shown), suggesting that the inhibitory mechanism of luteolin on NF-κB signaling is different in fibroblasts and macrophages.

Several reports have shown that the interaction between p65 and CBP is essential for NF-κB-dependent transcriptional activity [8,9]. CBP performs an important role in the integration of diverse signaling pathways by linking p65 to components of the basal transcriptional machinery, such as TFIIB, TBP, and histone acetyltransferase [9]. Our report demonstrated that luteolin inhibited the interaction of p65 with CBP and that this event was directly related to the inhibition of NF-κB transcriptional activity (Fig. 4A). Since CBP is present in limited amounts in the nucleus and is capable to interact with several transcription factors, competition for CBP provides another mechanism for transcriptional regulation [14–16]. Phosphorylated p65 associates with the CBP KIX domain (amino acids 452–661). The KIX domain is also the binding site for phosphorylated CREB and c-Jun [21–23]. We found that luteolin induced the phosphorylation of CREB and c-Jun (Fig. 4B and C). Moreover, the inhibition of p65·CBP complex formation and NF-κB transcriptional activity by luteolin was partially recovered by H89, a PKA inhibitor, which inhibited the phosphorylation of CREB and c-Jun by luteolin (Fig. 4). These results imply that phosphorylated CREB and c-Jun by luteolin can bind to the KIX domain of CBP, which limits the access of p65 translocated and phosphorylated in response to LPS. Therefore, we conclude that inhibition of NF-κB transcriptional activity by luteolin is caused by competition between transcription factors for limiting quantities of CBP.

In this report, we showed that the phosphorylation of CREB and c-Jun by luteolin was inhibited by H89 (Fig. 4B), implying that luteolin signaling may be involved in cyclic AMP/PKA pathway. Previous report has demonstrated that cAMP/PKA pathway inhibits NF-κB-mediated transcription without prevention of NF-κB nuclear translocation and phosphorylation [28]. Luteolin has its capacity to modulate cAMP level through inhibition of cAMP phosphodiesterase activity [29], which may explain the activation of cAMP/PKA pathway by luteolin. PKA can directly phosphorylate CREB, but not c-Jun. However, previous report has demonstrated that c-Jun phosphorylation is strongly enhanced by transfection of the catalytic subunit of PKA or forskolin treatment through an indirect activation mechanism [30]. Therefore, PKA activation by luteolin can phosphorylate both CREB and c-Jun. On the other hand, luteolin increased phosphorylation of CREB and c-Jun in Rat-1 cells, but not RAW 264.7 cells (Fig. 4B and C), implying cAMP/PKA pathway may be involved in such effect in different cells. However, more detailed studies will be required to investigate whether luteolin induces PKA activation through increasing intracellular cAMP level in Rat-1 cells.

In conclusion, this study showed that luteolin inhibited LPS-stimulated NF-κB transcriptional activity by modulating the transcription complex assembly in fibroblasts, suggesting a novel molecular mechanism for the regulation of NF-κB signaling by luteolin. Cell-type specificity of anti-inflammatory action of luteolin provides basis for the understanding of the regulation of fibroblasts and macrophages in inflammatory response.

Acknowledgments

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