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Full Paper

Turmeronols (A and B) from *Curcuma longa* have anti-inflammatory effects in lipopolysaccharide-stimulated BV-2 microglial cells by reducing NF-κB signaling

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Turmeronols (A and B), bisabolane-type sesquiterpenoids found in turmeric, reduce inflammation outside the brain in animals; however, their effects on neuroinflammation, a common pathology of various neurodegenerative diseases, are not understood. Inflammatory mediators produced by microglial cells play a key role in neuroinflammation, so this study evaluated the anti-inflammatory effects of turmeronols in BV-2 microglial cells stimulated with lipopolysaccharide (LPS). Pretreatment with turmeronol A or B significantly inhibited LPS-induced nitric oxide (NO) production; mRNA expression of inducible NO synthase; production of interleukin (IL)-1β, IL-6, and tumor necrosis factor α and upregulation of their mRNA expression; phosphorylation of nuclear factor-κB (NF-κB) p65 proteins and inhibitor of NF-κB kinase (IKK); and nuclear translocation of NF-κB. These results suggest that these turmeronols may prevent the production of inflammatory mediators by inhibiting the IKK/NF-κB signaling pathway in activated microglial cells and can potentially treat neuroinflammation associated with microglial activation.

Key words: *Curcuma longa* (turmeronol), neuroinflammation, microglial cell, lipopolysaccharide, nitric oxide, IκB kinase, nuclear factor-κB

INTRODUCTION

Curcuma longa L., also known as turmeric, is a member of the ginger family that is widely used as a traditional spice and has various physiological activities [1]. Turmeronols (A and B) are sesquiterpenoids that can be isolated from the dried rhizome and water extract of *C. longa* (Fig. 1) [2, 3]. Dried *C. longa* rhizome has been reported to contain turmeronols (A and B) at concentrations of about 200 µg/g and 300 µg/g, respectively [2]. Water extracts of *C. longa* have antioxidant and anti-inflammatory effects [3–6], increase the water content of skin [7], improve emotional fatigue [8], and have antidepressant effects [9]. A clinical study found that the daily intake of a water extract of *C. longa* containing turmeronols improves the levels of systemic inflammatory markers, such as C-reactive protein, and quality of life (QOL) scores on the 36-Item Short-Form Health Survey (SF-36) [10, 11]. However, the influence of turmeronols on neuroinflammation is not clearly understood.

Neuroinflammation, a central nervous system inflammatory response, can be triggered by infection, trauma, tissue injury, and neurotoxins [12] and involves glial cells [13], including microglial cells. It increases the permeability of the blood-brain barrier, recruits inflammatory cells into the target site to eliminate pathogens and dead cells [14], and promotes neuronal regeneration/repair [15]. Although these effects are beneficial in the short term, a chronic neuroinflammatory response, which is mediated by long-lived immune cells such as microglial cells, has various undesirable consequences, including neuronal

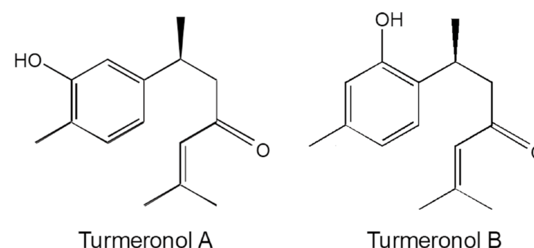

Fig. 1. Chemical structure of turmeronols.

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(Supplementary materials: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2480/)

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damage and dysfunction and poor tissue repair [12, 16]. Chronic neuroinflammation is known to contribute to the pathogenesis of mental diseases and various neurodegenerative diseases [17], such as Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, and Parkinson's disease [18].

Pro-inflammatory mediators such as nitric oxide (NO), interleukin (IL)-1β, IL-6, and tumor necrosis factor α (TNF-α) are produced by microglial cells in response to stimulation by conserved microbial structures, tissue damage signals, and abnormal proteins such as Aβ species. These mediators induce disruption of the blood-brain barrier, neuronal death, and synaptic loss [12, 19], all of which are involved in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease [20], Parkinson's disease [21], Huntington's disease [22], and amyotrophic lateral sclerosis [23]. Previous clinical studies showed microglial activation in patients with various neurodegenerative diseases [24]. Turmeronols were reported to have anti-inflammatory effects in RAW 264.7 macrophage cell lines [3], but the effects of turmeronols on the production of inflammatory mediators in microglial cells are not clearly understood.

To investigate the anti-neuroinflammatory effects of turmeronol A and turmeronol B, we measured the production and mRNA expression of inflammatory mediators and the protein expression of factors associated with inhibitor of nuclear factor kappa B (IκB) kinase (IKK)/nuclear factor kappa B (NF-κB) signaling in BV-2 mouse microglial cells stimulated with lipopolysaccharide (LPS).

MATERIALS AND METHODS

Cell culture

BV-2 cells (Interlab Cell Line Collection, Genova, Italy), an immortalized mouse microglial cell line, were seeded onto 24-well plates at 1.2×10^6 cells per well and incubated in 0.5 mL of Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) containing 10% heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT, USA), 100 U/mL penicillin (Sigma-Aldrich), and 100 µg/mL streptomycin (Sigma-Aldrich) for 24 hr at 37°C under an atmosphere of 5% CO_2. After removing the medium, cells were preincubated for 1 hr in 0.5 mL of FBS-free DMEM containing graded concentrations of turmeronol A or turmeronol B (Nagara Sciences, Gifu, Japan) and stimulated with LPS (from *Escherichia coli* O127:B8; product No. L3129, Sigma-Aldrich) for 6, 12 or 24 hr at a final concentration of 500 ng/mL. Then, cells at 6 hr or culture supernatants at 12 or 24 hr were collected for measurement of inflammatory mRNA or mediators. Lack of cytotoxicity of the test agents was confirmed in a preliminary experiment.

ELISA

In culture medium, all of ELISA kits were performed according to the manufacturer's instructions, including kits for IL-1β (R&D Systems, Rochester, MN, USA), TNF-α (R&D Systems), and IL-6 (Abcam, Cambridge, MA, USA).

Measurement of NO

The concentration of nitrite (a stable metabolite of NO) in culture supernatants was measured by the Griess method [25, 26].

Reverse transcription polymerase chain reaction (PCR)

Total RNA was extracted from the cultured cells with a Maxwell® RSC simplyRNA Cells Kit and automated RNA extraction system by using a Maxwell® RSC Instrument (Promega, Madison, WI, USA) according to the manufacturer's instructions [27–29]. The RNA was eluted with 50 µL of nuclease-free water. Then, expression of the inducible nitric oxide synthase (iNOS), IL-1β, IL-6, and TNF-α genes was investigated by reverse transcription PCR [30]. Complementary DNA synthesis and PCR were performed with a Thermal Cycler Dice Real Time System III (Takara code, TP970; Takara, Shiga, Japan) and One Step SYBR PrimeScript™ RT-PCR Kit II (Takara), respectively, according to the manufacturer's protocol. PCR primers were obtained from Fasmac (Kanagawa, Japan); the primer sequences are displayed in Supplementary Table 1. The $2^{-\Delta\Delta CT}$ method [31] was employed for data processing, which was based on analysis of the second derivative curve of amplified plots and performed with the Thermal Cycler Dice Real Time System software (version 6.01C, Takara). Target gene expression was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, which was confirmed to be stable by a preliminary analysis.

Preparation of a whole-cell extract and cytoplasmic and nuclear proteins

BV-2 cells were seeded into 6-well plates at 5.4×10^6 cells per well and incubated in 2 mL of DMEM (10% heat-inactivated FBS) for 18 hr. Then, the cells were pretreated with each test agent under serum-free conditions for 1 hr and stimulated with LPS (500 ng/mL) for 30 min. The cells were collected with a cell scraper (Techno Plastic Products AG, Trasadingen, Switzerland) and centrifuged at 300 *g* for 10 min at 4°C, after which the supernatant was removed. Cells were resuspended in ice-cold PBS and centrifuged at 300 *g* for 10 min at 4°C. To prepare the whole-cell extract, the pellet was lysed with ice-cold radioimmunoprecipitation assay (RIPA) buffer (Fujifilm Wako, Osaka, Japan) supplemented with cOmplete protease inhibitor cocktail tablets (Sigma-Aldrich) and PhosStop phosphatase inhibitor tablets (Sigma-Aldrich). After 1 hr on ice, the extract solution was centrifuged at 16,260 *g* for 20 min at 4°C, and the supernatant was collected. To prepare the cytoplasmic and nuclear proteins, the pellet was lysed with protein extraction buffer, and then cytosolic and nuclear proteins were extracted with an EzSubcell Extract kit (ATTO, Tokyo, Japan) according to the manufacturer's instructions. Total protein levels in the preparations were determined with a Micro BCA Protein Assay kit (Thermo Fisher Scientific, Vernon Hills, IL, USA).

Western blot analysis

Automated capillary electrophoresis-based Western blot analyses were performed on a ProteinSimple Wes® System with a 12-230 kDa Separation Module kit (ProteinSimple SM-W004, ProteinSimple, San Jose, CA, USA) and Anti-Rabbit Detection Module kit (ProteinSimple DM-001, ProteinSimple) according to the manufacturer's instructions [32–34]. In brief, samples were diluted to an appropriate concentration (0.02, 0.2, or 2 mg/mL) in 0.1 × sample buffer containing sodium dodecyl sulfate (SDS) and then combined with 5 × Fluorescent Master Mix (containing 5 × sample buffer, 5 × fluorescent standard, and 200 mM dithiothreitol) at a ratio of 4:1 and heated at 95°C for 5 min. The

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samples, blocking reagent (antibody diluent), primary antibodies, horseradish peroxidase (HRP)-conjugated secondary antibodies, and chemiluminescent substrate (luminol-S/peroxide) were added to each well of the microplate provided by the manufacturer. The primary anti-mouse antibodies against NF-κB p65, phospho-NF-κB p65 (Ser536), phospho-IKK-α/β (Ser176/180), lamin B1, and GAPDH were purchased from Cell Signaling Technology (Danvers, MA, USA); those against IKK-α/β were purchased from Abcam. The microplate was loaded into the instrument, which performed electrophoretic protein separation and immunodetection in the automated capillary system. The expression levels of GAPDH and lamin B1 were used as internal controls for cytoplasmic and nuclear proteins, respectively. These internal controls were confirmed to be stable by a preliminary experiment. The data were analyzed with the Compass software (version 4.1.0; ProteinSimple). Phosphorylated protein expression was expressed as the ratio of phosphorylated to total protein, and nuclear translocation of the NF-κB p65 subunit (as an indicator of NF-κB activation) was expressed as the ratio of nuclear to cytoplasmic NF-κB protein.

Statistical analysis

LPS-stimulated control cells and treated cells were compared with Student's t-test or Dunnett's multiple comparison test. Results are shown as the mean and standard deviation (SD). A probability (p) value of less than 0.05 was defined as indicating statistical significance, and analyses were performed with the Statcel 4 software (OMS Publishing, Tokorozawa, Japan).

RESULTS

Effects of turmeronol A and turmeronol B on NO production and synthetic enzyme mRNA expression in BV-2 cells stimulated with LPS

The NO level was markedly increased in LPS-stimulated control cells compared with unstimulated control cells, and LPS-induced NO production was significantly inhibited by pretreatment of cells with turmeronol A or turmeronol B (Fig. 2A). Expression of iNOS mRNA was upregulated by LPS stimulation, and this upregulation was significantly suppressed by pretreatment of cells with turmeronol A or turmeronol B (Fig. 2B).

Effects of turmeronol A and turmeronol B on inflammatory cytokine production and mRNA expression in BV-2 cells stimulated with LPS

LPS stimulation markedly increased IL-1β, IL-6, and TNF-α production, but the increase was inhibited by pretreatment of cells with turmeronol A or turmeronol B (Fig. 3A–3C). Expression of IL-1β, IL-6, and TNF-α mRNA was also markedly upregulated by LPS stimulation, and this upregulation was significantly inhibited by pretreatment with turmeronol A or turmeronol B (Fig. 3D–3F).

Effects of turmeronol A and turmeronol B on phosphorylation of IKK-α/β in BV-2 cells stimulated with LPS

The phosphorylation of IKK-α/β protein (Ser176/180) was increased in control cells after 30 min of LPS stimulation, but it was inhibited by pretreatment with turmeronol A or turmeronol B (Fig. 4).

Effects of turmeronol A and turmeronol B on nuclear translocation of NF-κB in BV-2 cells stimulated with LPS

The nuclear level of p65 protein was markedly increased in control cells after 30 min of LPS stimulation, and this was inhibited by pretreatment with turmeronol A or turmeronol B (Fig. 5A and 5B). These results suggested that turmeronol A and turmeronol B inhibited translocation of NF-κB from the cytoplasm to the nucleus after 30 min of LPS stimulation.

Effects of turmeronol A and turmeronol B on phosphorylation of NF-κB p65 in BV-2 cells stimulated with LPS

The phosphorylation of NF-κB p65 (Ser536) was markedly increased in control cells after 30 min of LPS stimulation and inhibited by pretreatment with turmeronol A or turmeronol B (Fig. 6A and 6B).

DISCUSSION

In the present study, pretreatment with turmeronol A or turmeronol B significantly inhibited NO production and mRNA expression of iNOS in BV-2 mouse microglial cells stimulated with LPS. The turmeronols also significantly inhibited LPS-induced production and mRNA expression levels of IL-1β, IL-6, and TNF-α. In addition, both turmeronols significantly suppressed

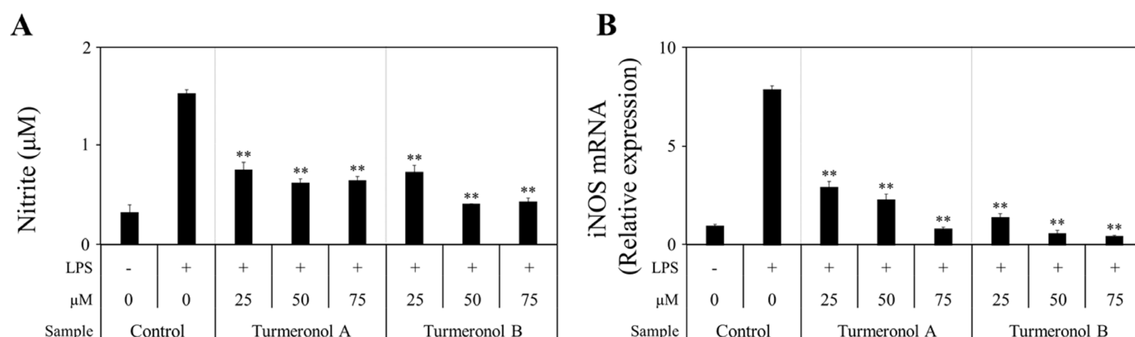

Fig. 2. Effects of turmeronol A and turmeronol B on NO production and mRNA expression of synthetic enzymes in BV-2 cells stimulated with LPS.

Cells were preincubated with turmeronol A or turmeronol B for 1 hr under serum-free conditions and subsequently stimulated with LPS (500 ng/mL) for (A) 12 hr or (B) 6 hr. NO levels in culture supernatants were measured by the Griess assay. Inducible NO synthase mRNA expression was determined by reverse transcription polymerase chain reaction with glyceraldehyde-3-phosphate dehydrogenase as an internal control. Data are expressed as the mean ± standard deviation (n=3). **p<0.01 vs. LPS-stimulated control cells (Dunnett's test).

iNOS: inducible nitric oxide synthase; LPS: lipopolysaccharide; NO: nitric oxide; LPS: lipopolysaccharide.

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the increase in phosphorylated IKK and NF-κB proteins and the nuclear translocation of NF-κB. These results suggest that the turmeronols may prevent the production of inflammatory mediators by reducing the activation of IKK/NF-κB signaling in activated microglial cells.

Microglial cells play an important role in immune surveillance of the central nervous system by removing harmful pathogens and cellular debris [18]. However, excessive microglial activation by inflammatory stimuli such as pathogen-associated molecular patterns and damage-associated molecular patterns leads to production of high amounts of pro-inflammatory cytokines and neurotoxic factors, which cause neural dysfunction [12]. In addition, clinical studies have identified microglial activation as a pathological hallmark of neuroinflammation in patients with various neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and primary progressive multiple sclerosis [24]. In animal models of neurodegenerative diseases, neuroinflammation was improved by minocycline, a commonly used inhibitor of microglial activation [35]. In the present study, both turmeronols prevented the increase in protein and mRNA expression levels of various inflammatory mediators in LPS-activated microglial cells (Figs. 2 and 3), indicating that they may potentially ameliorate the neuroinflammation mediated by the activation of microglial cells.

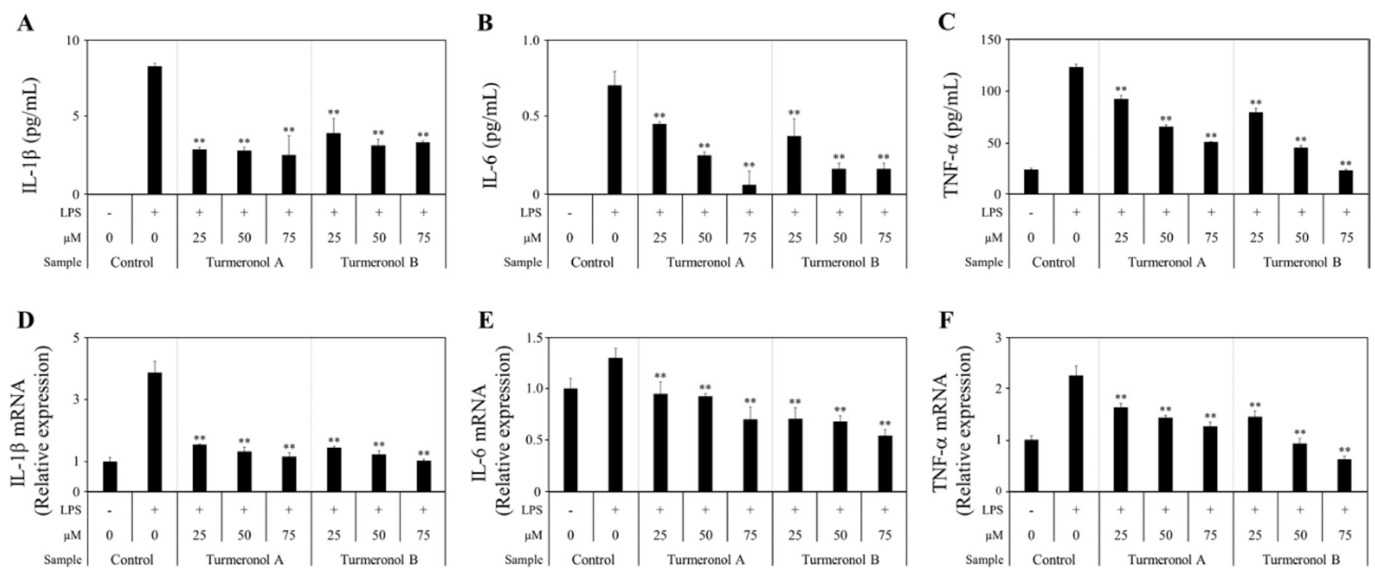

Fig. 3. Effects of turmeronol A and turmeronol B on inflammatory cytokine production and mRNA expression in BV-2 cells stimulated with LPS.

Cells were preincubated with turmeronol A or turmeronol B for 1 hr under serum-free conditions and subsequently stimulated with LPS (500 ng/mL) for (A–C) 24 hr or (D–F) 6 hr. (A) IL-1β, (B) IL-6, and (C) TNF-α protein levels in culture supernatants were measured by enzyme-linked immunosorbent assay. (D) IL-1β, (E) IL-6, and (F) TNF-α mRNA expression was determined by reverse transcription polymerase chain reaction with glyceraldehyde-3-phosphate dehydrogenase as an internal control. Data are expressed as the mean ± standard deviation (n=3). **p<0.01 vs. LPS-stimulated control cells (Dunnett's test).

IL: interleukin; LPS: lipopolysaccharide; TNF: tumor necrosis factor.

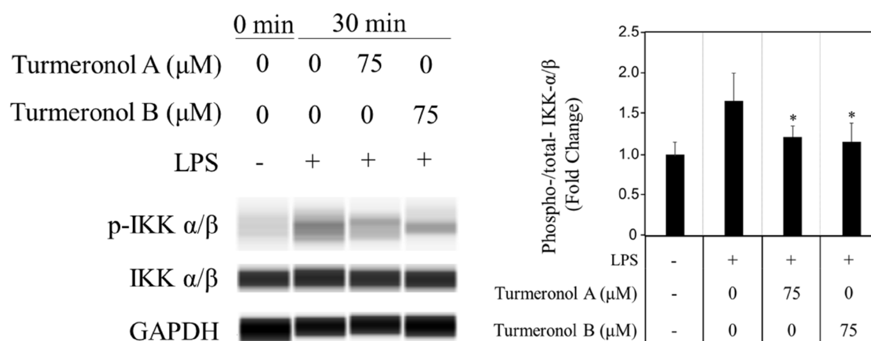

Fig. 4. Effects of turmeronol A and turmeronol B on phosphorylation of IKK-α/β in BV-2 cells stimulated with LPS.

Cells were preincubated with turmeronol A or turmeronol B for 1 hr under serum-free conditions and subsequently stimulated with LPS (500 ng/mL) for 30 min. After preparation of whole cell extracts, IKK-α/β protein expression was determined by Western blotting with GAPDH as an internal control protein. The relative band intensity of phosphorylated IKK-α/β was normalized to that of total IKK-α/β and expressed in the graph. Data are expressed as the mean ± standard deviation (n=3). *p<0.05 vs. LPS-stimulated control cells (Dunnett's test).

GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IKK: inhibitor of nuclear factor kappa B kinase; LPS: lipopolysaccharide.

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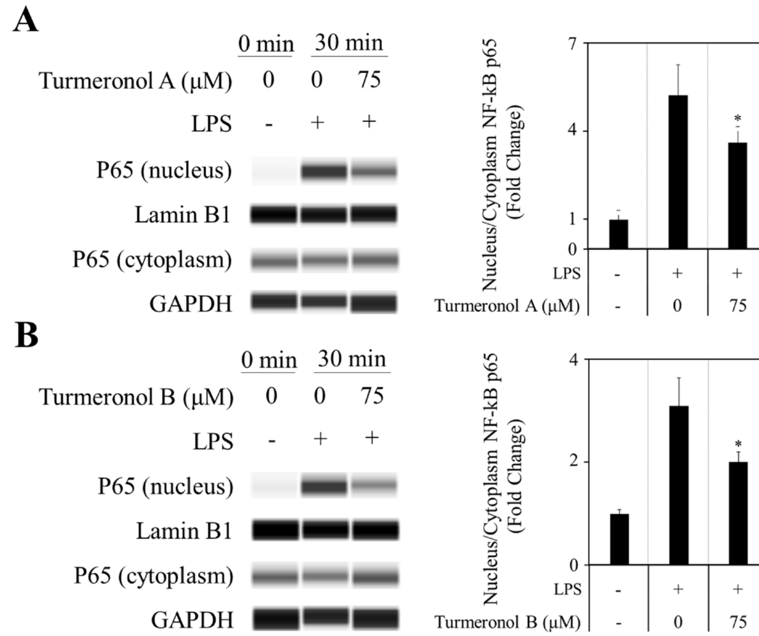

Fig. 5. Effects of turmeronol A and turmeronol B on nuclear translocation of NF-κB in cytoplasmic and nucleus protein extracts of BV-2 cells stimulated with LPS.

Cells were preincubated with turmeronol A or turmeronol B for 1 hr under serum-free conditions and subsequently stimulated with LPS (500 ng/mL) for 30 min (A and B). After preparation of cytoplasmic and nuclear cell extracts, NF-κB p65 protein expression was determined by Western blotting with GAPDH and lamin B1 as a cytoplasmic and nuclear internal control protein, respectively. For the graph, the relative band intensity of nuclear NF-κB p65 was normalized to that of cytoplasmic NF-κB p65. Data are expressed as the mean ± standard deviation (n=3–6) of three independent experiments. *$p<0.05$ vs. LPS-stimulated control cells (Student's t-test).

GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPS: lipopolysaccharide; NF-κB: nuclear factor kappa B.

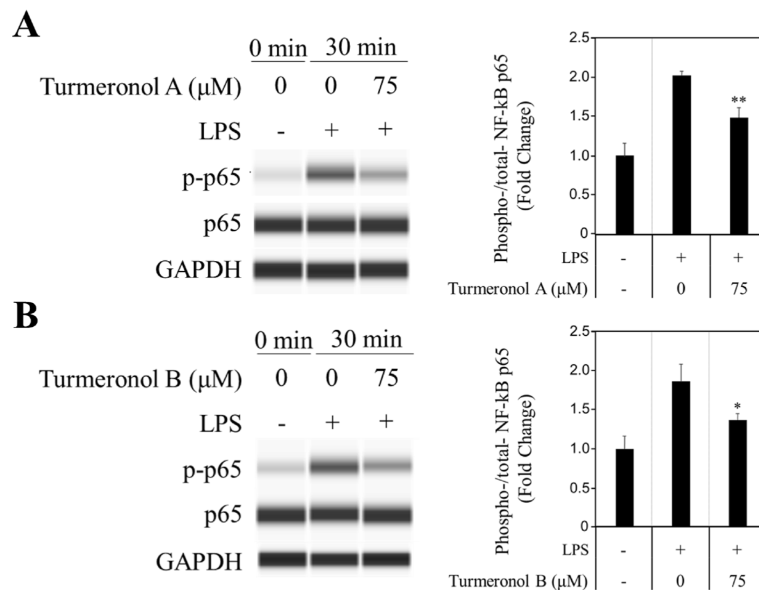

Fig. 6. Effects of turmeronol A and turmeronol B on phosphorylation of NF-κB in BV-2 cells stimulated with LPS.

Cells were preincubated with turmeronol A or turmeronol B for 1 hr under serum-free conditions and subsequently stimulated with LPS (500 ng/mL) for 30 min (A and B). After preparation of whole cell extracts, NF-κB p65 protein expression was determined by Western blotting with GAPDH as the internal control protein. For the graph, the relative band intensity of phosphorylated NF-κB p65 was normalized to that of total NF-κB p65. Data are expressed as the mean ± standard deviation (n=3). *$p<0.05$, **$p<0.01$ vs. LPS-stimulated control cells (Student's t-test).

GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPS: lipopolysaccharide; NF-κB: nuclear factor kappa B.

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Pro-inflammatory mediators produced by a variety of cell types, including microglial cells, are known to have undesirable effects in the central nervous system [18, 36]. NO interacts with superoxide to form ONOO-, which induces neuronal cell death by causing oxidative damage to cellular components such as DNAs, lipids, and proteins [19]. IL-1β and TNF-α have also been shown to cause neuronal death by activating caspase-8 and increasing synaptic loss [12]. IL-6 has been reported to promote Aβ-induced neurotoxicity and to increase the protein expression of vascular cell adhesion molecule 1 and gene expression of iNOS [37, 38]. A study in a mouse model of Alzheimer's disease showed that depletion of either TNF-α receptor 1 or iNOS genes inhibits synaptic disruption and memory impairment [39]. In addition, research in Parkinson's disease mouse models showed that IL-1 or IL-6 deficiency prevents microglial activation and behavioral impairment [40, 41]. In the present study, both turmeronols significantly suppressed the production of NO and inflammatory cytokines in microglial cells (Figs. 2 and 3). Taken together, these results suggest that the turmeronols may prevent neuronal cell death and dysfunction by inhibiting the production of inflammatory mediators.

IKK, an enzyme complex consisting of three subunits (IKK-α, IKK-β, and IKK-γ), is essential for activation of NF-κB signaling pathways [42]. The binding of LPS to toll-like receptor 4 can recruit cytoplasmic adaptor proteins such as myeloid differentiation primary response 88, which in turn phosphorylates the IKK complex. Activated IKK-β increases the degradation of IκB-α and allows NF-κB translocation from the cytoplasm to the nucleus, and activated NF-κB induces the expression of target genes, including iNOS, IL-1β, IL-6, and TNF-α [43, 44, 45]. In agreement with previous studies, LPS stimulation in the present study increased phosphorylation of IKK-α/β and translocation of NF-κB from the cytoplasm to the nucleus (Figs. 4 and 5). In another study, pretreatment with Compound A—an IKK-β selective inhibitor—suppressed mRNA expression levels of inflammatory mediators such as NO, IL-1β, and TNF-α in rat primary microglial cells stimulated with LPS [46]. In the present study, both turmeronols significantly reduced LPS-induced phosphorylation of IKK-α/β and nuclear translocation of NF-κB (Figs. 4 and 5). These results suggest that the turmeronols may inhibit expression of inflammatory mediator genes by reducing activation of the IKK/NF-κB signaling pathway.

Transcription of NF-κB target genes is dependent on not only nuclear translocation of NF-κB but also phosphorylation of NF-κB p65. Phosphorylated NF-κB p65 is known to enhance its transcriptional activity by promoting acetylation at the lysine residue of p65 and interaction with cAMP-response element-binding protein (CREB)-binding protein (CBP)/p300, a transcriptional co-activator protein [47, 48]. In fact, licochalcone A, a component of licorice root, has been shown to suppress phosphorylation of NF-κB p65 and mRNA expression of NF-κB target genes, including iNOS, TNF-α, and MCP-1, without inhibiting nuclear translocation of NF-κB [49]. In addition, NF-κB p65 at Ser536 is reported to be phosphorylated by some protein kinases, including IKK-α and IKK-β [47]. In the present study, both turmeronols reduced phosphorylation of IKK and NF-κB p65 proteins in BV-2 cells stimulated with LPS (Fig. 6). Taken together, these findings raise the possibility that turmeronols reduce the increase in the levels of phosphorylated NF-κB p65 protein by inhibiting phosphorylation of IKK.

The molecular targets of turmeronols (A and B) are not clearly understood. Parthenolide, one of the sesquiterpenoids in the anti-inflammatory medicinal herb *Tanacetum parthenium*, has been reported to inhibit the activation of NF-κB and IKK by directly binding to cysteine residue at position 179 (Cys-179), a critical step in IKK-β enzymatic activity [50, 51]. In addition, arsenite and cyclopentenone prostaglandins, IKK-β inhibitors, also target this cysteine residue [52, 53]. The replacement of Cys-179 by an alanine residue of the IKK-β protein is known to block the IKK-β inhibitors that bind to this protein [51–53], resulting in reduced phosphorylation of activation loop serine, which is required for IKK-β activation [54]. Thus, turmeronols may also reduce IKK activation by binding to IKK-β at Cys-179; however, further research is needed to clarify whether turmeronols can directly bind to IKK-β.

The blood-brain barrier (BBB) limits transportation between peripheral circulation and the central nervous system. It plays an important role in protecting the central nervous system from harmful agents and microorganisms in the blood [55]. To date, it remains unclear whether turmeronols (A and B) can cross the BBB. However, α-, β-, and aromatic-turmerone, all of which have a sesquiterpene structure similar to that of the turmeronols contained in *C. longa*, were detected not only in serum but also in the brains (cerebrum and cerebellum) of mice administered these turmerones [56–58]. Thus, turmeronols may cross the BBB like other sesquiterpenes and influence microglial cells in the central nervous system. Further research on this topic is needed.

In animal models of neurodegenerative diseases, the pathology of neuroinflammation in the central nervous system was improved by functional food ingredients that inhibit production of inflammatory mediators in LPS-stimulated BV-2 microglial cell lines [59–61]. In addition, microglial inhibitors, including minocycline and a selective colony stimulating factor 1 receptor inhibitor, were reported not only to suppress neuroinflammation in animal models but also to improve cognitive function and the antidepressant effect of minocycline [35, 62–65]. Clinical intervention studies showed that a hot water *C. longa* extract, which contains turmeronols, significantly improves serum levels of inflammatory markers, such as C-reactive protein, IL-6, and TNF-α, and mental health scores on the SF-36 questionnaire [10, 11]. Therefore, turmeronol A and turmeronol B may be effective for improving neuroinflammation-related symptoms. To confirm the results of our *in vitro* experiments, the effects of turmeronols on neuroinflammation should be evaluated *in vivo* in two animal models of neurodegenerative diseases, i.e., the amyloid precursor protein (APP) transgenic mouse model of Alzheimer's disease [66, 67] and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease [68], because minocycline, a commonly used inhibitor of microglial activation, showed neuroprotective effects in both these models [69, 70].

In conclusion, we found that pretreatment with either turmeronol A or turmeronol B inhibited the production of inflammatory mediators and their gene expression levels in microglial BV-2 cells activated with LPS. In addition, both turmeronols reduced phosphorylation of IKK and NF-κB p65 and nuclear translocation of NF-κB. These results suggest that turmeronol A and turmeronol B have the potential to prevent neuroinflammation induced by activated microglial cells.

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AUTHORSHIP

The authors' contributions were as follows: R.S., R.U., A.F., C.O.-H., K.K., K.M., S.M., Y.Y., and Y.H. designed the study; R.S., R.U., and A.F. conducted the *in vitro* study; R.S., R.U., and A.F. analyzed data; R.S., R.U., A.F., K.K., and K.M participated in interpretation of the results; R.S., R.U., C.O.-H., K.M., S.M., and Y.H. wrote the manuscript; and Y.H. had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

We declare that we have no conflicts of interest.

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