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Berberine Suppresses Neuroinflammatory Responses Through AMP-Activated Protein Kinase Activation in BV-2 Microglia

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ABSTRACT

The AMPK cascade is a sensor of cellular energy change, which monitors the AMP/ATP ratio to regulate cellular metabolism by restoring ATP levels, but its regulation of neuroinflammation mechanism remains unclear. Berberine, one of the major constituents of Chinese herb Rhizoma coptidis, has been shown to improve several metabolic disorders, such as obesity and type II diabetes. However, the effect of berberine on neuroinflammatory responses in microglia are poorly understood. This study shows that berberine represses proinflammatory responses through AMP-activated protein kinase (AMPK) activation in BV-2 microglia. Our findings also demonstrate that berberine significantly down-regulates LPS- or interferon (IFN)-γ-induced nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2) expression in BV-2 microglia cells. Berberine also inhibited LPS- or IFN-γ-induced nitric oxide production. In addition, berberine effectively inhibited proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 expression. On the other hand, upon various inflammatory stimulus including LPS and IFN-γ, berberine suppressed the phosphorylated of ERK but not p38 and JNK in BV-2 microglia. AMPK activation is catalyzed by upstream kinases such as LKB1 and Ca2+/calmodulin-dependent protein kinase kinase-II (CaMKK II). Moreover, berberine induced LKB1 (Ser428), CaMKII (Thr286), and AMPK (Thr172) phosphorylation, but not AMPK (Ser485). Furthermore, the inhibitory effect of berberine on iNOS and COX-2 expression was abolished by AMPK inhibition via Compound C, an AMPK inhibitor. Berberine-suppressed ERK phosphorylation was also reversed by Compound C treatment. Our data demonstrate that berberine significantly induces AMPK signaling pathways activation, which is involved in anti-neuroinflammation. J. Cell. Biochem. 110: 697–705, 2010.

KEY WORDS: LPS; IFN-Γ; GLIA; AMPK; NEUROINFLAMMATION

Microglia are thought to belong the monocyte/macrophage lineage of cells that reside in the brain parenchyma and have been proposed to play a role in host defense and tissue repair in the central nervous system. Under pathological conditions, activated microglia have been implicated as the predominant cell type governing inflammation-mediated neuronal damage. Upon chronic activation, microglia are capable of producing a variety of proinflammatory mediators and potentially neurotoxic compounds. Although these immunotoxic factors are necessary for normal function, the microglia response must be tightly regulated to avoid overactivation and disastrous neurotoxic consequences [Hudson et al., 2002]. Overactivation of microglial cells may cause severe brain tissue damage in various neurodegenerative diseases [Kreutzberg, 1996]. Glial activation involves changes in cell phenotype and the expression of new proteins, such as iNOS and COX-2. The mechanism by which activated glia induce neuronal death has been shown to involve nitric oxide [Loihl and Murphy, 1998; Bal-Price and Brown, 2001], reactive oxygen species (ROS) and proinflammatory cytokines [Beckman et al., 1994; Chao et al., 1995]. LPS and IFN-γ potently increases the levels of cellular MAP kinases phosphorylation, thereby promoting proinflammatory responses in microglia. Consistently, specific inhibition of cellular

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MAP kinase suppresses inflammatory signaling, implying that the cellular regulator for MAP kinase activity might be a key factor for inflammatory responses.

AMPK is a intracellular energy sensor implicated in the regulation of glucose and lipid homeostasis [Hardie et al., 1998; Zang et al., 2004], and is activated by phosphorylation on threonine-172 (Thr172) of the α-subunit in response to a decrease in cellular energy charge and a fall in ATP to AMP ratios [Leclerc and Rutter, 2004]. AMPK is a heterotrimeric complex composed of a catalytic α-subunit and regulatory β- and γ-subunits, and is allosterically activated by the phosphorylation of Thr172 on the α-subunit, which is catalyzed by upstream kinases such as LKB1 and CaMKK II [Hawley et al., 2005; Woods et al., 2005]. In addition, various cellular and metabolic stresses such as glucose deprivation, exercise, and hypoxia also induce AMPK activation [Hardie, 2003; Hardie and Sakamoto, 2006]. 5-Aminoimidazole-4-carboxamide-1-β-ribofuranoside (AICAR) is widely used as a pharmacologic activator of AMPK [Hardie et al., 1998]. In agreement with its protective activation from metabolic stresses, AMPK has been proposed as an anti-apoptotic molecule to protect cell injury resulting from glucose deprivation [Culmsee et al., 2001], hyperglycemia [Ido et al., 2002], and ischemia [Russell et al., 2004]. On the other hand, some reports indicate the ability of AMPK to inhibit inflammation of macrophage, microglia and astrocytes [Giri et al., 2004; Saitoh et al., 2004], however, the function of AMPK in anti-neuroinflammation remains not clear.

Berberine, one of the major constituents of Chinese herb Rhizoma coptidis, has been used to treat type II diabetes in China. Recent reports have shown that berberine activates AMPK in adipocytes, muscle, endothelial cell, and liver [Jeong et al., 2009; Kim et al., 2009; Lamontagne et al., 2009; McCarty et al., 2009; Wang et al., 2009], which contributes to its beneficial metabolic effects in these tissues. Additionally, it has been also reported that berberine suppresses the expression of inflammatory molecules in several cell types [Kuo et al., 2004; Choi et al., 2006; Kim et al., 2007; Lee et al., 2007; Hu et al., 2008]. In the present study, we address the questions whether berberine down-regulates inflammatory responses and how berberine exhibits anti-inflammatory responses in microglia. Here, we demonstrate that berberine reduces the expression of proinflammatory mediators upon LPS- and IFN-γ stimuli in an AMPK-dependent manner.

MATERIALS AND METHODS

MATERIALS

Berberine was purchased from Sigma–Aldrich (St. Louis, MO). AICAR was purchased from Calbiochem (San Diego, CA). Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), and OPTI-MEM were purchased from Gibco-BRL (Invitrogen Life Technologies, Carlsbad, CA). Primary antibodies against β-actin, ERK2, and phospho-ERK1/2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibodies against AMPK phosphorylated at Thr172, and Ser485, CaMKII phosphorylated at Thr286, and LKB1 phosphorylated at Ser428 were purchased from Cell Signaling and Neuroscience (Danvers, MA). Compound C was purchased from Calbiochem. All other chemicals were obtained from Sigma–Aldrich.

CELL CULTURE

The murine BV-2 cell line was cultured in DMEM with 10% FBS at 37°C in a humidified incubator in an atmosphere of 5% CO_2 and 95% air. Confluent cultures were passaged by trypsinization.

WESTERN BLOT ANALYSIS

BV-2 cells were treated with berberine for indicated time periods and then washed with cold PBS that had been lysed for 30 min on ice with radioimmunoprecipitation assay buffer (50 mM HEPES (PH 7.4), 150 mM NaCl, 4 mM EDTA, 10 mM $Na_4P_2O_7$, 100 mM NaF, 2 mM Na_3VO_4, 1% Triton X-100, 0.25% sodium deoxycholate, 50 mM 4-(2-aminoethyl) benzene sulfonylfluoride, 50 μg/ml leupeptin, and 20 μg/ml aprotinin). Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat milk in PBS for 1 h at room temperature and then probed with primary antibodies. After undergoing three PBS washes, the membranes were incubated with secondary antibodies. The blots were visualized by enhanced chemiluminescence using Kodak X-OMAT LS film (Eastman Kodak, Rochester, NY). Quantitative data were obtained using a computing densitometer and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

REVERSE TRANSCRIPTION AND QUANTITATIVE REAL TIME-PCR

Total RNA was extracted from BV-2 cells using a TRIzol kit (MDBio, Inc., Taipei, Taiwan). The reverse transcription reaction was performed using 2 μg of total RNA that was reverse transcribed into cDNA. Quantitative real-time PCR using SYBR Green I Master Mix was analyzed with a model 7900 Sequence Detector System (Applied Biosystems, Foster City, CA). After preincubation at 50°C for 2 min and 95°C for 10 min, the PCR was performed as 40 cycles of 95°C for 10 s and 60°C for 1 min. The threshold was set above the non-template control background and within the linear phase of target gene amplification to calculate the cycle number at which the transcript was detected (denoted as C_T). The oligonucleotide primers were iNOS: 5′-CCCAGAGTTCCAGCTTCTGG-3′ and 5′-CCAAGCCCCTCACCATTATCT-3′; COX-2: 5′-TGGGGTGATGAGCAACTATT-3′ and 5′-AAGGAGCTCTGGGTCAAACT-3′; IL-1β: 5′-TGGGGGAGATTCTCACTTTG-3′ and 5′-CCATCAGCGTTCCCATACTT-3′; IL-6: 5′-CCAGTTGCCTTCTTGGGACTG-3′ and 5′-CAGGTCTGTTGGGAGTGGTATCC-3′; TNF-α: 5′-AAAATTCGAGTGACAAGCCTGTAG-3′ and 5′-CCCTTGAAGAGAACCTGGGAGTAG-3′; GAPDH: 5′-CTCAACTACATGGTCTACATGTTCCA-3′ and 5′-CTTCCCATTCTCAGCCTTGACT-3′.

STATISTICS

Statistical analysis was performed using software Graphpad Prism 4.01 (Graph Pad Software, Inc., San Diego, CA). The values given are means ± SEM. Statistical analysis between two samples was performed using Student's *t*-test. Statistical comparisons of more than two groups were performed using one-way ANOVA with Bonferroni's post hoc test. In all cases, a value of $P < 0.05$ was considered as significant.

RESULTS

BERBERINE SUPPRESSES LPS- AND IFN-Γ-INDUCED iNOS AND COX-2 EXPRESSION IN BV-2 MICROGLIA

We used BV-2 microglia cell line to study the anti-neuroinflammatory mechanism of berberine. To determine the effect of berberine on iNOS and COX-2 protein levels, cells were pretreated with various concentrations of berberine for 30 min and then stimulated with LPS (100 ng/ml) for 24 h. As shown in Figure 1A,B, berberine significantly inhibited LPS-induced iNOS and COX-2 expression concentration-dependently. To further confirm the anti-inflammatory effect of berberine on BV-2 microglia, cells were pretreated with berberine and then stimulated with IFN-γ (10 μg/ml) for 24 h. Berberine also inhibited IFN-γ-induced iNOS (Fig. 1C) and COX-2 (Fig. 1D) production in a concentration-dependent manner. The cell culture medium was then harvested to determine the nitrite content by Griess reaction. The results showed that LPS or IFN-γ increases the production of nitric oxide by approximately ninefold. Berberine effectively antagonized LPS- (Fig. 1E) or IFN-γ (Fig. 1F)-induced nitric oxide production. Berberine at a concentration up to 10 μM by itself did not affect the release of nitric oxide. Berberine at concentrations ranging from 1 to 10 μM did not affect cell viability using MTT assay (data not shown). Next, we examined the potential of the inhibitory effect of berberine on iNOS and COX-2 expression after LPS induction. The cells were incubated with LPS for 30–120 min to induce iNOS and COX-2 expression, and then treated with berberine until 24 h. Post-treatment of berberine also decreased LPS-induced iNOS and COX-2 expression (Fig. S1).

BERBERINE SUPPRESSES THE LPS- AND IFN-Γ-INDUCED PROINFLAMMATORY CYTOKINE EXPRESSION

We further examined the effect of berberine on LPS- or IFN-γ-induced mRNA expression of proinflammatory cytokines, including IL-6, TNF-α, and IL-1β using real-time PCR analysis. Total RNA were extracted from BV-2 microglia after LPS stimulation for 6 h. Pretreatment with berberine decreased LPS-induced proinflammatory cytokine production (Fig. 2A). In addition, berberine also antagonized IFN-γ-induced proinflammatory cytokine expression (Fig. 2B). These data suggest that berberine effectively inhibited LPS- or IFN-γ-induced iNOS, COX-2, and proinflammatory cytokine expression.

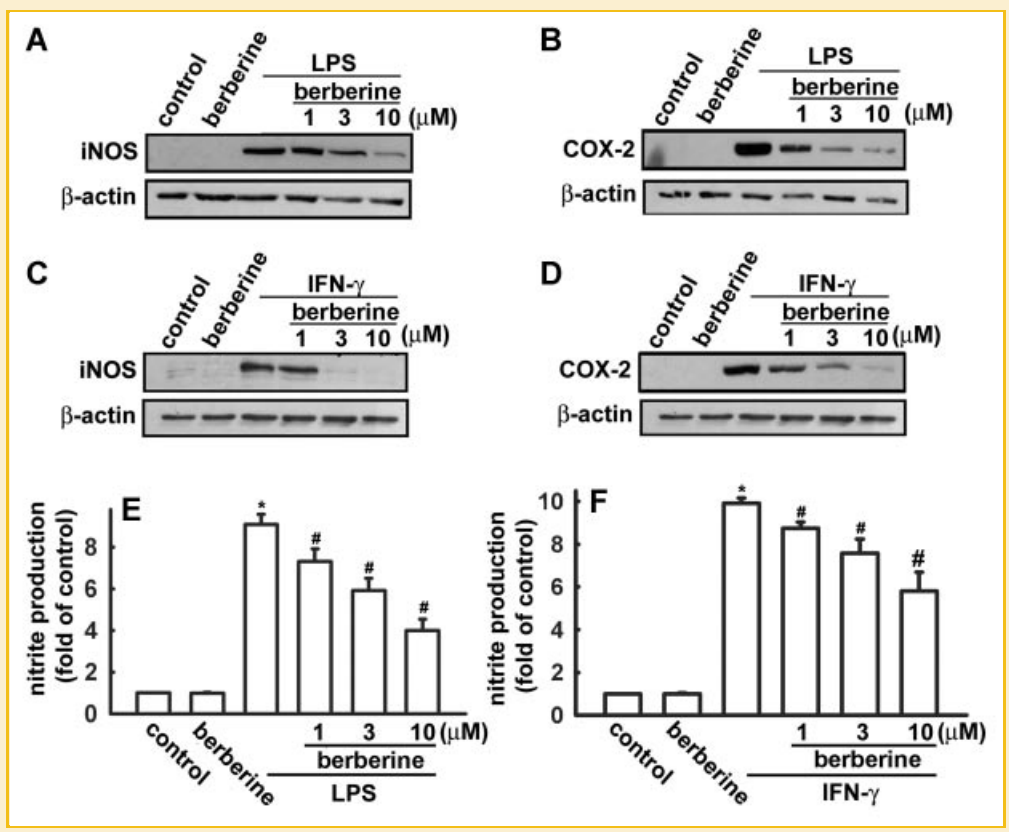

Fig. 1. Berberine suppresses iNOS and COX-2 expression in BV-2 microglia cells. Cells were pretreated with various concentrations of berberine (0, 1, 3, or 10 μM) for 30 min followed by stimulation with LPS (100 ng/ml) or IFN-γ (10 μg/ml) for 24 h. Whole cell lysis protein were extracted and iNOS (A,C) and COX-2 (B,D) protein levels were determined using Western blot analysis. The cell culture medium was then harvested to determine the nitrite content by Griess reaction (E,F). The nitrite content is significantly different between berberine treatment group and LPS, or IFN-γ alone group (one-way ANOVA followed by Bonferroni's post hoc test). The data represent the mean ± SEM of n = 3–4. $^{*}P < 0.05$ compared with the control group. $^{\#}P < 0.05$ compared with the LPS or IFN-γ treatment.

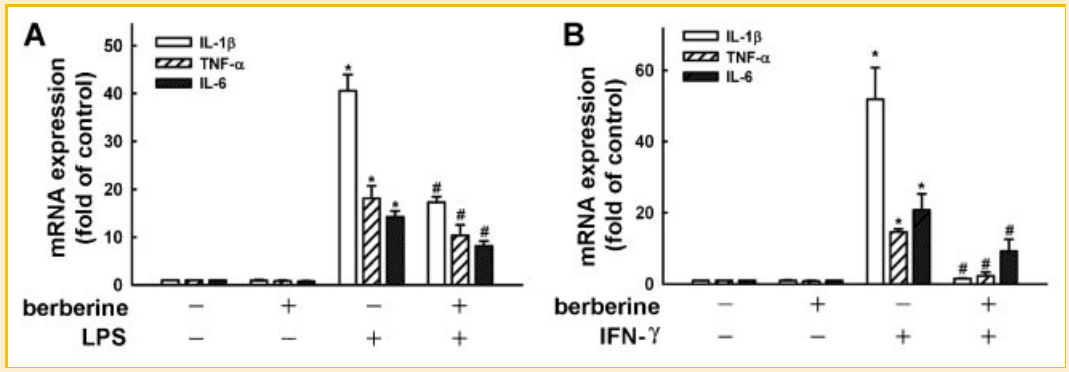

Fig. 2. Inhibitory effects of berberine on proinflammatory cytokine expression in BV-2 microglia cells. BV-2 cells were pretreated with berberine (10 μM) for 30 min followed by stimulation with LPS (100 ng/ml) or IFN-γ (10 μg/ml) for 6 h. Relative mRNA levels of IL-1β, TNF-α, and IL-6 were analyzed by real-time PCR and normalized with GAPDH. Each bar represents means ± SEM of n = 4–5. $^{*}P < 0.05$ compared with the control group; $^{\#}P < 0.05$ compared with the LPS or IFN-γ treatment.

BERBERINE INHIBITS ERK PHOSPHORYLATION BUT NOT P38 AND JNK

MAP kinase signal molecules are among the most important molecules in the signaling pathways that control the synthesis and release of proinflammatory mediators by activated macrophage and microglia [Koistinaho and Koistinaho, 2002; Wang et al., 2002; Jang et al., 2005]. Previous report has showed that berberine effectively antagonized LPS-induced MAP kinases phosphorylation, such as p38, ERK, and JNK [Jeong et al., 2009]. In addition, we examined whether these kinases activation are modulated by berberine in BV-2 cells. Cells were pretreated with different concentrations of berberine for 30 min and then stimulated with LPS or IFN-γ for 60 min. Whole cell lysis protein were extracted and subjected to Western blot for phospho-ERK, phospho-p38 or phospho-JNK. As shown in Figure 3A,B, berberine effectively inhibited the ERK activation, however, berberine up to 10 μM did not affect LPS- or IFN-γ-induced p38 and JNK phosphorylation.

BERBERINE STIMULATES AMPK SIGNALING PATHWAYS ACTIVATION IN BV-2 MICROGLIA

Previous studies have shown that berberine stimulates AMPK activation in macrophage, myotubes, and hepatocytes [Brusq et al., 2006; Cheng et al., 2006; Lee et al., 2006; Jeong et al., 2009]. Next, we examined the effect of berberine on AMPK activation, which is directly linked with its phosphorylation state. We treated BV-2 cells with berberine and examined the levels of AMPK phosphorylation. As shown in Figure 4A, berberine enhanced AMPK phosphorylation at Thr172 site but not Ser485 site. Furthermore, berberine also increased upstream molecular of AMPK, LKB1 (Ser428), and CaMKII (Thr286) phosphorylation within a short-term period 30 min and

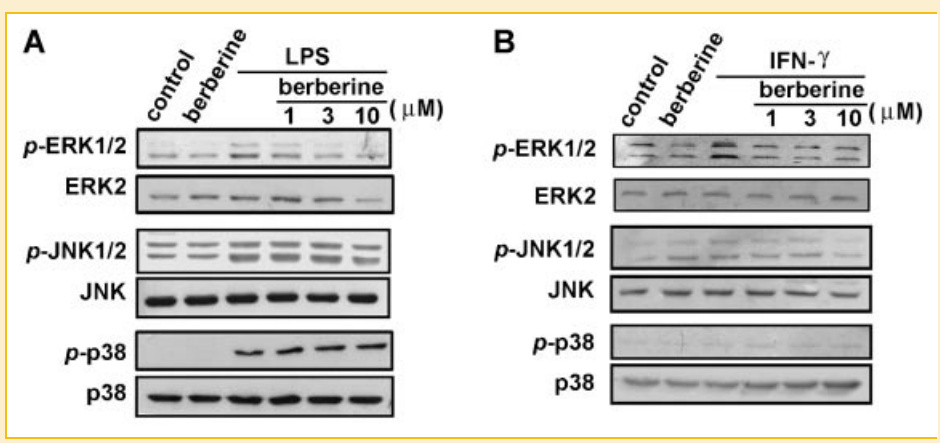

Fig. 3. Effects of berberine on LPS or IFN-γ-induced MAP kinases activation in BV-2 microglia. Cells were preincubated with various concentrations of berberine (0, 1, 3, or 10 μM) for 30 min, and then stimulated with LPS (100 ng/ml; A) or IFN-γ (10 μg/ml; B) for 60 min. Total cell lysates were subjected to Western blot for phospho-ERK, phospho-p38, or phospho-JNK. Similar results were obtained from at least four independent experiments. Relative phosphorylation levels of MAP kinases were normalized by each total protein.

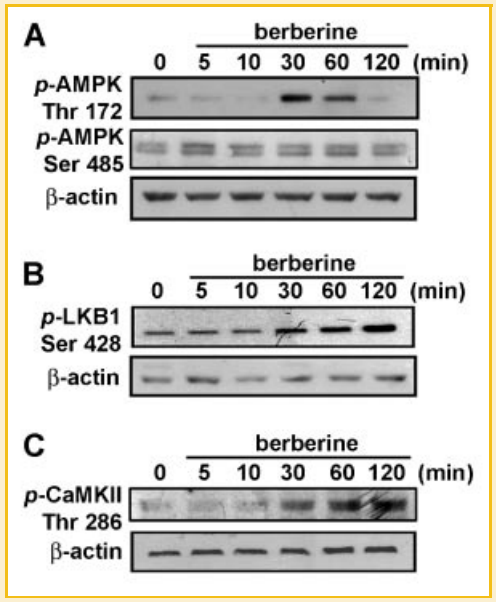

Fig. 4. Phosphorylation of AMPK signaling pathways by berberine in BV-2 microglia cells. Cells were incubated with berberine (10 μM) for indicated time periods (0–120 min). Whole cell lysates were subjected to Western blot analysis using antibodies against the phosphorylation of the AMPK α-subunits at Thr172 and Ser485 (A), LKB1 (Ser428) (B) and CaMKII (Thr286) (C). Similar results were obtained from at least four independent experiments.

prolonged its phosphorylated state at 2 h (Fig. 4B,C). These results demonstrate that berberine activates AMPK signaling pathways in BV-2 microglia.

AMPK INHIBITION ATTENUATES THE REPRESSIVE EFFECTS OF BERBERINE ON INFLAMMATORY RESPONSES

A recent report has shown that berberine suppresses proinflammatory responses through AMPK activation in macrophages [Jeong et al., 2009]. We further examined whether AMPK activation is required for the inhibitory effects of berberine on proinflammatory responses. To this end, we utilized Compound C, an AMPK inhibitor, to suppress the cellular AMPK activity. In the presence of Compound C, the inhibitory effects of berberine on LPS-induced iNOS and COX-2 expression were alleviated in BV-2 microglia (Fig. 5A,B). In addition, Compound C also antagonized the inhibitory action of berberine on IFN-γ-induced iNOS (Fig. 5C) but not COX-2 expression (Fig. 5D). Furthermore, the cell culture medium was then harvested to determine the nitrite content by Griess reaction. In the presence of Compound C, the inhibitory effects of berberine on LPS- or IFN-γ-induced nitric oxide production were completely abrogated in BV-2 microglia (Fig. 5E,F). Therefore, our results and previous studies imply that berberine-induced AMPK activation might be an important step in mediating the anti-inflammatory responses. To further explore whether AMPK activation is also involved in the down-regulation of ERK activation by berberine, cells were pretreated with Compound C followed with berberine. As shown in Figure 6, inhibition of AMPK by Compound C also alleviated the suppressive effects of berberine on ERK phosphorylation upon LPS and IFN-γ stimulation.

Furthermore, we also confirmed the effect and molecular mechanisms of 5-aminoimidazole-4-carboxamide riboside (AICAR; a pharmacologic activator of AMPK) in LPS- or IFN-γ-related iNOS and COX-2 expression in BV-2 cells. As shown in Figure 7, cells pretreated with AICAR (1 mM) antagonized LPS- or IFN-γ-induced iNOS and COX-2 expression. On the other hand, AICAR also induced AMPK phosphorylation at Thr172 and Ser485 time-dependently (Fig. 7E). Taken together, these results reveal that berberine-dependent AMPK activation would play a crucial role in down-regulating neuroinflammatory responses through suppression of inflammatory signaling cascades including ERK signal.

DISCUSSION

AMPK has been shown to play a critical role in the regulation of energy metabolism under both physiological and pathological conditions. A main aim of the present study is to elucidate the contribution of the AMPK signaling pathways in the attenuation of cytokines expression induced by LPS or IFN-γ in BV-2 microglia cells. Our results demonstrate that (1) berberine effectively inhibits LPS- or IFN-γ-induced iNOS, COX-2, and proinflammatory cytokine expression; (2) berberine inhibits iNOS and COX-2 expression, and ERK activation through AMPK activation; (3) berberine also induces AMPK upstream, LKB1, and CaMKII activation; (4) berberine activates AMPK through AMPKα Thr172 but not Ser485 phosphorylation in BV-2 microglia cells. Recent studies have shown that berberine ameliorates metabolic disorders via multiple pathways including the decrease of inflammatory mediator expression [Kuo et al., 2004; Brusq et al., 2006; Choi et al., 2006; Lee et al., 2006, 2007; Kim et al., 2007]. However, the molecular mechanism by which berberine represses proinflammatory responses in microglia remains unclear. In this study, we demonstrate that berberine potently inhibited proinflammatory responses through AMPK activation.

MAP kinases are stimulated by many inflammatory stimuli, including LPS, IFN-γ, and TNF-α. Furthermore, activation of the three MAP kinases (ERK1/2, p38, and JNK) are linked with induction of iNOS expression in microglia [Wang et al., 2002; Moon et al., 2007]. However, it is unclear which MAP kinases critically play a key role to turn on proinflammatory gene expression in microglia. Here, we observed that various inflammatory stimuli, including LPS and IFN-γ, elevated the phosphorylation of MAP kinases, such as ERK1/2, p38, and JNK signaling pathways. Recent report has showed that berberine inhibits LPS-induced MAP kinase phosphorylation of ERK p38, and JNK [Jeong et al., 2009]. However, berberine has also been reported to have a lipid lowering effect by stabilizing hepatic LDL receptor mRNA in an ERK-dependent manner rather than promoting transcriptional activity [Abidi et al., 2005]. Moreover, it is also reported that berberine suppresses MEK/ERK-dependent signaling pathway to inhibit vascular smooth muscle cell regrowth [Liang et al., 2006]. Here, we find that berberine represses the phosphorylation of ERK signal induced by LPS and IFN-γ. We also observed that an inhibitory effect of berberine on ERK

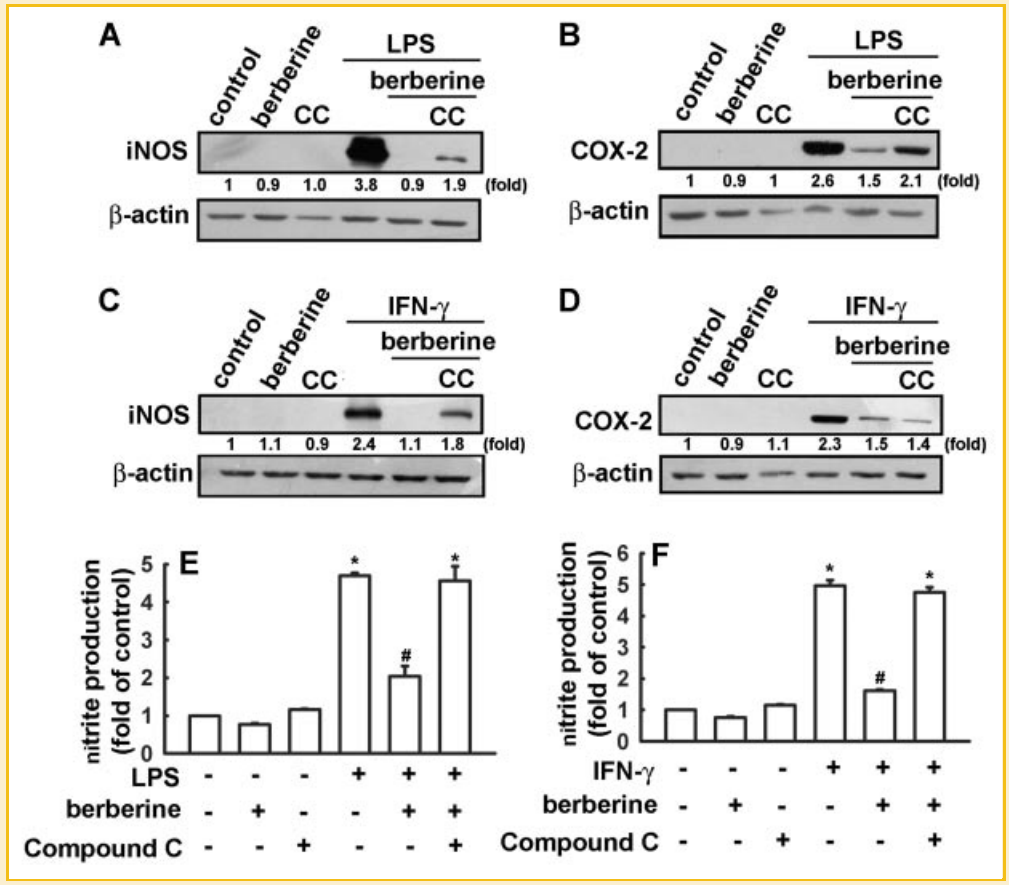

Fig. 5. Involvement of AMPK in the inhibition of berberine on LPS- or IFN-γ-induced iNOS and COX-2 expression. BV-2 cells were pretreated with Compound C (10 μM) for 30 min and treated with berberine (10 μM) for another 30 min before LPS (100 ng/ml) or IFN-γ (10 μg/ml) treatment. Whole cell lysis protein were extracted and subjected to Western blot for iNOS (A,C) and COX-2 (B,D) after 24 h incubation with LPS or IFN-γ, and the culture medium was then collected for the assay of nitrite (E,F). Each bar represents means ± SEM of n = 4–5. $^{*}P < 0.05$ compared with the control group; $^{\#}P < 0.05$ compared with the LPS or IFN-γ treatment. Similar results were obtained from at least four independent experiments.

activation through AMPK activation, leading to inhibitory effects of berberine on inflammatory cytokine expression. Therefore, the inhibition of MAP kinase involved in berberine treatment might be dependent on cell types and experimental condition. Notably, numerous reports have also shown that berberine suppresses IL-1β and TNF-α production via the inhibition of NF-κB signaling pathway [Hsiang et al., 2005; Lee et al., 2007; Ho et al., 2009]. Recently, it have shown that berberine has no effect on leptin-induced STAT-3 phosphorylation [Choi et al., 2009]. Here we found that berberine also antagonizes LPS-induced IκBα activation and IFN-γ-induced STAT-1 and STAT-3 phosphorylation (Fig. S2).

Although many inflammatory diseases are associated with increased levels of IFN-γ and COX-2, the relationship between IFN-γ, COX-2, and inflammation is not well understood. A previous

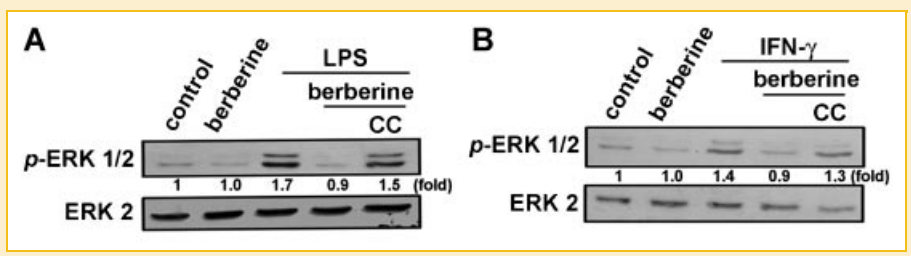

Fig. 6. Effects of berberine on AMPK inhibition in LPS- or IFN-γ-induced ERK phosphorylation. BV-2 cells were pretreated with Compound C (10 μM) for 30 min and incubated with berberine (10 μM) for another 30 min before LPS (100 ng/ml, A) or IFN-γ (10 μg/ml, B) treatment for 60 min. Whole cell lysis protein were extracted and subjected to Western blot for phospho-ERK. Similar results were obtained from at least four independent experiments.

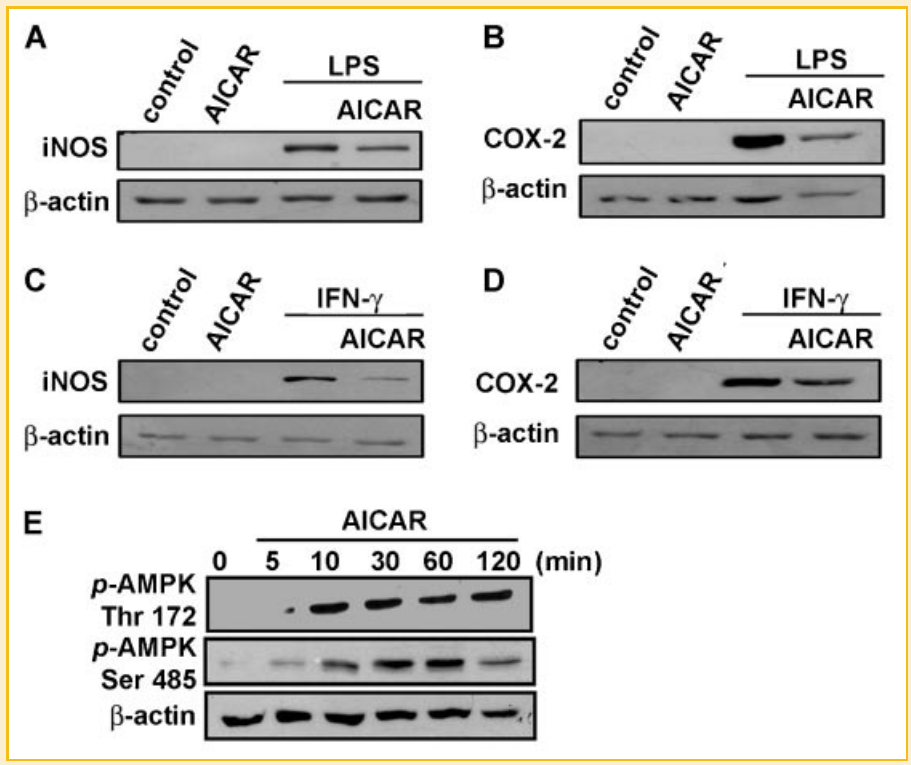

Fig. 7. Effects of AICAR in LPS- or IFN-γ-induced iNOS and COX-2 expression. BV-2 cells were pretreated with AICAR (1 mM) for 30 min before LPS (100 ng/ml) or IFN-γ (10 μg/ml) treatment. Whole cell lysis protein were extracted and subjected to Western blot for iNOS (A,C) and COX-2 (B,D) after 24 h incubation with LPS or IFN-γ. E: Cells were incubated with AICAR (1 mM) for indicated time periods (0–120 min). Whole cell lysates were subjected to Western blot analysis using antibodies against the phosphorylation of the AMPK α-subunits at Thr172 and Ser485. Similar results were obtained from at least 4 independent experiments.

study demonstrated that IFN-γ-induced COX-2 expression and -increased PGE_2 production is regulated by CRE/ATF site in the COX-2 promoter region in human epidermal keratinocyte cells [Matsuura et al., 1999]. It has also been demonstrated that IFN-γ-induced COX-2 expression through a member of the IRF family, IRF-1, but not the transcriptional factors including NF-κB, C/EBP, CREB, and AP-1 in RAW 264.7 cells [Uto et al., 2007]. Moreover, it has also been reported that PKC-α negatively modulates IFN-γ-induced COX-2 expression in RAW 264.7 cells [Giroux and Descoteaux, 2000]. Here, we do not observe AMPK reverses the inhibitory effect of berberine on IFN-γ-induced COX-2 expression. We suggest there may be multiple mechanism involved in the induction of COX-2 by IFN-γ in addition to the MAP kinase pathways. The function of AMPK in metabolism has been well studied, however, its roles in anti-neuroinflammation remains not clear. It might depend on cell types, cellular events following external stimuli, duration of AMPK activation.

The key step in the activation of AMPK is mediated through its major regulatory phosphorylation site, threonine 172 (Thr172) [Hardie, 2003]. Recent studies have identified LKB1 and CaMKK as kinases that phosphorylate AMPK Thr172 [Carling et al., 2008]. Here we discuss the role of LKB1 and CaMKK in the regulation of AMPK and the two upstream kinases. Our results exhibited that berberine promoted CaMKII (Thr286), LKB1 (Ser428), and AMPK (Thr172) phosphorylation in BV-2 microglia. On the other hand, AMPK Ser485 in the α-subunit is an auto phosphorylation site as well as a target site for AKT [Horman et al., 2006]. Phosphorylation of Ser485 by AKT also regulated the AMPK activity. Furthermore, it has been reported that down-regulation of AMPK activity by cAMP-elevating reagents is associated with the phosphorylation of Ser485 in the α-subunit [Hurley et al., 2006]. We thus evaluated Ser485 phosphorylation in response to the inhibitory effects of berberine. Our data indicated that berberine induces AMPKα Thr172 phosphorylation but not Ser485, and might through activated LKB1 (Ser428) and CaMKII (Thr286). Our results suggest that AMPK Thr172 phosphorylation site might be an important anti-inflammation therapeutic target. Our finding also suggests that berberine might be useful as a therapeutic agent for the treatment of neuroinflammation associated with disorders.

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