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Inhibitory effect of berberine on interleukin-2 secretion from PHA-treated lymphocytic Jurkat cells

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ABSTRACT

Berberine is an isoquinoline alkaloid isolated from herb plants, such as *Cortex phellodendri* (Huangbai) and *Rhizoma coptidis* (Huanglian). Huanglian and Huangbai have been used as “heat-removing” agents. In addition, berberine has been reported to exert anti-inflammatory effect both *in vivo* and *in vitro*, where mitogen-activated protein kinase (MAPK) and cyclooxygenase-2 (COX-2) expressions are critically implicated. We herein tested the hypothesis that berberine exerts an anti-inflammatory effect through MAPK and COX-2 signaling pathway in T-cell acute lymphoblastic leukemia (T-ALL). In Jurkat cells, we found that PHA exposure caused elevation on interleukin-2 (IL-2) production in a time-dependent manner. PHA-stimulated reactions were steeply suppressed by berberine, such as IL-2 mRNA expression and protein secretion. However, berberine did not exert any cytotoxic effect at doses of 40 μg/ml. In addition, the possible molecular mechanism of anti-inflammation effect of berberine could be the inhibition of PHA-evoked phosphorylation of p38, since c-Jun N-terminal kinases (JNK) and extracellular signal-regulated kinase (ERK) expressions did not alter. Consistent with above results, berberine inhibition on PHA-induced IL-2 secretion could be reversed by treatment of SB203580, a specific inhibitor of p38-MAPK. Interestingly, upregulation of PHA-induced COX-2 expression was also observed following berberine treatment of Jurkat cells. Furthermore, flow cytometry analysis showed berberine-induced cell cycle arrest at G1 phase after PHA stimulation and decreased percentage of G2/M phase. In conclusion, our study demonstrated that the anti-inflammatory effect of berberine largely potentially results from its ability to attenuate p38 MAPK expression, and does not exclude a positive action of berberine on cell cycle arrest. These results provide an innovative medicine strategy to against or treat T-ALL patients.

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1. Introduction

The main feature of T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological cancer that arises from T-cell progenitors. Clinical studies have shown that ALL patients are accompanied with strong inflammatory state, owing to elevated cytokine levels in circulation system [1,2]. Intensified chemotherapy is the most common treatment schedules for T-ALL, but often combined with considerable side effects [3,4]. Despite the progress of chemotherapy in recent years, > 50% of adult patients with T-ALL still appear resistance to therapy and relapsed/refractory phenomenon [5], and the 5-year survival rate is as low as 45% for adult T-ALL patients [6,7]. T-ALL has therefore been rated the most challenging issue of malignant tumor, especially with respect to therapy. Hence, to find new effective therapies, especially herb drugs, for T-ALL patients are still a goal for researchers.

Berberine, a quaternary ammonium of isoquinoline alkaloids, is purified from medicinal plants, such as *Rhizoma coptidis* (Huanglian) and *Hydrastis canadensis* (goldenseal) [8]. Berberine has traditionally been used as herbal medicine in Asia, including China. Previous studies showed that berberine displays potential anti-inflammation activities by suppressing mitogen-activated protein kinase (MAPK) and promoting macrophage apoptosis [9]. Moreover, with respect to cancer biology, MAPK signaling pathway plays a critical role in cell growth/proliferation process. There is a growing body of evidence supporting the inhibitory effects of berberine on tumorigenicity of numerous types of cancer cells, by attribute of modulating cell growth-associated mechanisms, including MAPK signaling pathway [10,11] and apoptosis-inducing proteins [12,13]. On the other hand, cyclooxygenase-2 (COX-2), an enzyme that catalyzes prostaglandin biosynthesis, is one of the most common proteins correlated with inflammation and tumorigenesis. However, studies on the role of COX-2 expression in cancer biology remain controversial. COX-2 has been shown to have both positive and negative effects on cell proliferation. Investigators have showed that decreased COX-2 is associated with anti-cancer activity in leukemia cell through several mechanisms. It not only promotes cell apoptosis and cell cycle arrest [14], but attenuates tumor cell blastogenesis as well [14,15]. In contrast, overexpression of COX-2 in osteosarcoma cells leads to dramatic cell number decrease by 50%–70%, and attenuates proliferation with cell cycle continuation in G2-M [16]. Therefore, it is necessary to clarify the cancer cell growth phenomenon by COX-2 expression. With respect to pharmacological activities, berberine exerts a tumor-suppressive effect in part by attenuating COX-2 expression and has been linked to not only the attenuation of cell proliferation [17,18] but inflammatory responses [19]. We have thus hypothesized that berberine could diminish T-ALL inflammatory properties and cell blastogenesis by down-regulating MAPK and COX-2.

The evidence is crystal clear that berberine is able to treat various cancers and attests a close relevance between MAPK and COX-2 signaling pathways. Although the anti-inflammation and anti-tumor properties of berberine on T-ALL are concerned, not much is revealed on the subject. In current study, the effects of berberine on inflammatory molecules and cell cycle were investigated by observing the impact of berberine on corresponding pathways, including MAPK and COX-2, in Jurkat cells, which is a T-cell acute lymphoblastic leukemia cell.

2. Methods and materials

2.1. Materials

Enzyme-linked immunosorbent assay (ELISA) kits and antibodies for measuring IL-2 were obtained from R&D Systems (Minneapolis, MN, USA). Antibodies against phospho-c-Jun N-terminal kinases (p-JNK), phospho-extracellular signal-regulated kinase (p-ERK), phospho-p38, COX-2, β-actin and secondary antibodies were purchased from Cell Signaling (Beverly, MA, USA). The lactate dehydrogenase (LDH) assay kit was purchased from Abcam (Cambridge, MA, USA). Chemical reagents (L-glutamine, sodium pyruvate, sodium bicarbonate, HEPES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The following materials were ordered from the companies indicated: berberine, phytohemagglutinin (PHA, Sigma-Aldrich), cell culture media (RPMI-1640) and fetal bovine serum (Gibco, Green Island, NY, USA).

2.2. Cell culture

T-ALL Jurkat cells were routinely cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 10 mM HEPES at 37 °C in a humidified chamber containing 5% CO_2. T-ALL Jurkat cells were grown in 24-well culture plates at a density of 1×10^6 cells/ml. This study evaluated the inflammatory properties in the presence (treatment group) and absence (placebo group) of berberine in T-ALL Jurkat cells. As illustrated in the text and figure legends, cells were cultured in berberine and PHA at different time intervals depending on subsequent measurements.

2.3. IL-2 ELISA assay

Determination of IL-2 level was done following the method described previously [20]. Briefly, polystyrene microtiter plates (NUNC, U16 Maxisorp type, Denmark) were coated with capture antibodies (R&D Systems) at room temperature overnight. The next day, IL-2 standard and samples were added to the plate wells, incubated at room temperature after blocked, and exposed to detection antibody (R&D Systems) followed by streptavidin horseradish peroxidase enzyme (R&D Systems). IL-2 concentrations were measured using a tetramethylbenzidine substrate and the reaction was ceased with 2 N sulphuric acid. Finally, absorbance at 450 nm was read on a plate reader (BioTek, Winooski, VT, USA). Cytokine standard curves were used to calculate the amount of IL-2 in supernatant samples. The IL-2 ELISA was established with a detection range from 31.5 to 2000 pg/ml, and the intra- and inter-assay coefficients of variation were 10.2% and 6.4%, respectively.

2.4. RNA isolation and reverse transcription-PCR

Total RNA from Jurkat cells were extracted using Trizol Reagent kit (ThermoFisher Scientific, Grand Island, NY, USA) according to manufacturer's instructions. Total RNA was reverse-transcribed into single-stranded cDNA by superscript III pre-amplification system. IL-2 gene expression carried out a multiplex polymerase chain reaction kit for human sepsis cytokines set (Maxim Biotech, San Francisco, CA, USA) following standard procedures. The sense and antisense primer sequences of PCR primers were as follows: IL-2, forward primer 5′-ACC TCAACTCCTGCCACAAT-3′, reverse primer 5′-GCACTTCCTCCAGAGG TTTG-3′, and GAPDH, forward primer 5′-GAGTCAACGGATTTGG CGT-3′, reverse primer 5′-GACAAGCTTCCCGTTCTCAG-3′. PCR amplifications were performed in a thermal cycler (Thermolyne, Dubuzue, IA, USA), and the cycling conditions were as follows: 35 cycles (IL-2) and 30 cycles (GAPDH) of denaturation at 94 °C for 30 s, annealing at 52 °C for 60 s, and extension at 72 °C for 60 s. The amplified cDNA were analyzed through ethidium bromide staining following electrophoresis on agarose gel.

2.5. Cytotoxicity measurements

We discovered that soluble cytoplasmic lactate dehydrogenase (LDH) leakage into the culture medium from Jurkat cells indicated undergoing or arisen cell damage. The assessment of cytotoxic properties of berberine on Jurkat cells was performed by measuring the LDH leakage. In this experiment, Jurkat cells were seeded in plates at a concentration of 4×10^5 cells/well and exponentially growing cells were pre-treated with berberine (40 μg/ml) for 1 h, then stimulated with PHA for 24 h. Upon post-treatment, media and cell lysates were collected for LDH evaluation. The assays were carried out in triplicate by LDH cytotoxicity detection kit, according to manufacturer's instructions. The optical density was measured at 339 nm with a plate reader (Beckman, Westbury NY, USA).

2.6. Western blotting analysis

The method for immunoblotting has been described elsewhere [21,22] with some modification. Cells were pre-incubated with berberine for 1 h and subsequently stimulated with PHA for 15 min. Jurkat cell extracts were prepared with whole cell lysis buffer containing 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 20 mM Na2HPO4, 100 mM NaCl, 20 mM NaF, 0.2 mM PMSF, and 1 mM DTT. Aliquots of protein lysates were loaded into sodium dodecyl sulfate polyacrylamide gel, and after electrophoresis, transferred to polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA) with a glycine transfer buffer, followed by blocked with 3% bovine serum albumin in tris-buffered saline/0.1% Tween 20. The membrane was subsequently probed with the primary antibodies, followed by reaction with horseradish peroxidase–conjugated corresponding secondary antibody. All blots were developed using a chemiluminescence assay (Amersham International PLC, Buckinghamshire, UK), and exposed to X-ray film to visualize the bands. β-actin served as the loading control.

2.7. Cell cycle analysis with flow cytometry

Jurkat cells were seeded in 10-cm dish at a density of 3×10^4 cells/ml for 24 h and washed twice with 1× ice-cold PBS. The exponentially growing Jurkat cells were treated with berberine at concentration of 10 and 20 for 1 h and subsequently stimulated with PHA (1 μg/ml) for 30 min. After incubation, the cells were treated with trypsin-EDTA (0.25%) for 1 min, resuspended in 10% FBS RPMI-1640 media, and then centrifuged at 1000 rpm for 5 min. Cell pellets were fixed with 70% ethanol, centrifuged for 10 min, and then incubated with PBS containing Triton X-100 and ribonuclease. The resuspended cells were put in 37 °C water bath for 1 h, centrifuged for 5 min, and stained with propidium iodide in PBS. Cell cycle analysis was executed on the flow cytometry machine (FACSC alibus E6147; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Data was analyzed and quantified using BD cellQuestTM Pro 4.02 program. The experiments were done at least three times with similar results.

2.8. Statistical analysis

SPSS statistical software for Windows (SPSS Inc., Chicago, Ill., U.S.A.) was carried out for data statistical analysis. All results were performed one-way analysis of variance (ANOVA) followed by *Post hoc* analysis using Duncan's multiple range test. In other cases, Student's *t*-test was employed. Any statistical differences set at $P < 0.05$ were considered crucial. All the experiments were repeated and analyzed at least three times with equivalent results.

3. Results

3.1. Improvement of IL-2 secretion by PHA in activated Jurkat T cells

PHA is a well-known stimulator for lymphocyte function. However, the optimal dosage and the time period exerted through PHA on T-ALL activity in our laboratory is still unknown. We first investigated whether PHA stimulated IL-2 secretion from T-ALL Jurkat cells by exposing cultured Jurkat cells to PHA (5 μg/ml) at different time periods. Supernatant was harvested and IL-2 concentrations were evaluated by ELISA. PHA administration of Jurkat T cells produced a remarkable

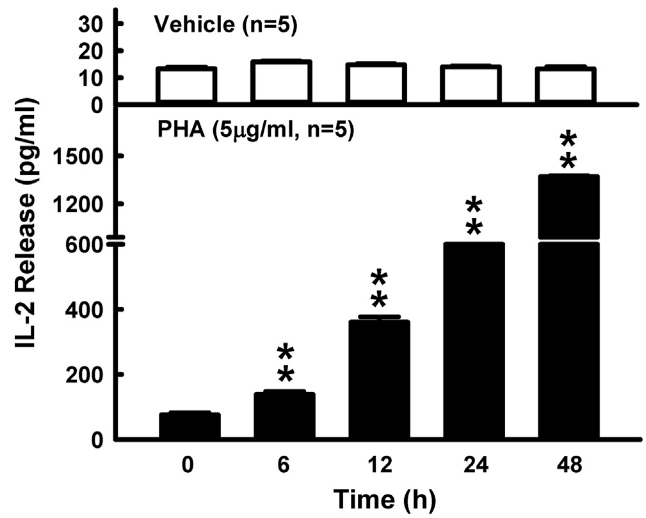

Fig. 1. Evaluation of IL-2 level following stimulation with PHA in Jurkat cells. Jurkat cells (1×10^6 cells/ml) were treated with PHA for 6, 12, 24, and 48h. *IL-2* concentration was measured by ELISA. Data were expressed as mean ± SEM. ** $P < 0.01$ indicates significant differences compared with 0 h group.

enhancement of IL-2 production, which was time-dependent (6, 12, 24, and 48 h), when compared to 0-h group (Fig. 1). Hence, 5 μg/ml of PHA was selected for stimulation Jurkat cells for 24 h in the following experiment.

3.2. The effects of berberine, upon PHA-mediated IL-2 suppression

To assess the inhibitory effects of berberine on PHA-evoked IL-2 secretion, IL-2 production from PHA-stimulated Jurkat cells was measured in the presence of berberine. As shown in Fig. 2A, berberine steeply attenuated IL-2 production in a dose-dependent manner up to 20 μg/ml, while berberine did not inhibit IL-2 production under the vehicle conditions. To confirm the aforementioned results, IL-2 expression in response to PHA in the presence or absence of berberine was further investigated. As illustrated in Fig. 2B, PHA elicited a significant promotion on the expression of IL-2 mRNA in Jurkat cells. In contrast, berberine supplementary restored the gene expression of PHA-evoked IL-2 as compared to the levels observed in PHA-treated only group. The above results suggested that berberine strongly blunted IL-2 production from T-ALL Jurkat cells.

3.3. The intracellular and media LDH activities in Jurkat cells treated with berberine and PHA

To examine the possible toxicity of berberine on T-ALL Jurkat cells, lactate dehydrogenase (LDH) activity was used as a cytotoxicity marker. LDH assay represents membrane integrity and is a direct detection of cell death. The cytotoxic effect of berberine (40 μg/ml) on Jurkat cells is shown in Fig. 3. There was no significant difference in LDH leakage of cytoplasm and media between PHA- and berberine-treated Jurkat cells. This result suggested that IL-2 production from PHA-induced Jurkat cells was diminished by berberine at a range from 40 μg/ml without cytotoxic effect.

3.4. Inhibition of p38 kinase phosphorylation in response to berberine in Jurkat cells with PHA

Previous studies demonstrated that phosphorylation of MAPK (ERK1/2, JNK1/2, and p38) plays an important role in different cell types. The expression of IL-2 is mediated by pathways that involved MAPKs in T lymphocytes [23,24]. To investigate the possible signaling pathways responsible for anti-inflammatory effects of berberine *in vitro*,

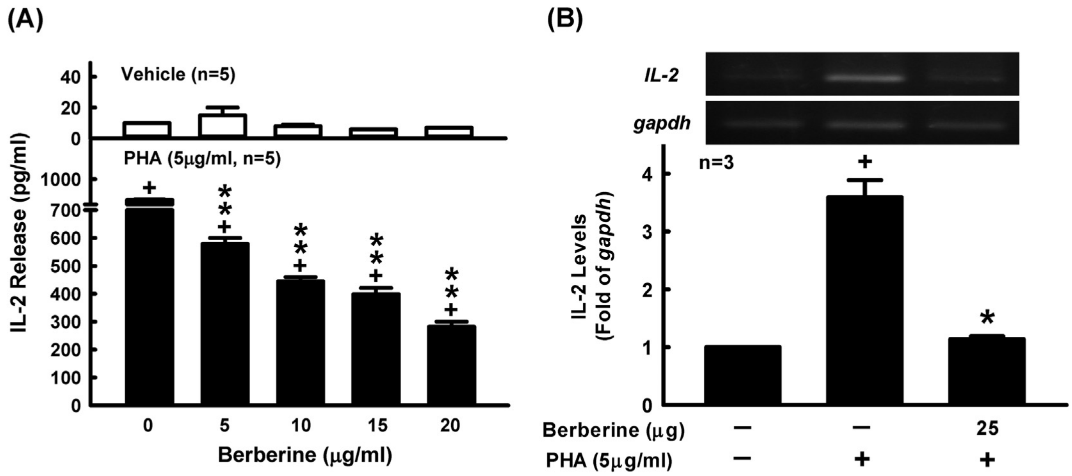

Fig. 2. Berberine attenuates IL-2 levels in response to PHA in Jurkat cells. (A) Jurkat cells were untreated (vehicle) or stimulated with berberine (5, 10, 15, 20 μg/ml) with or without the addition of PHA (5 μg/ml) for 24 h. Subsequently, the extent of IL-2 secretion was determined by ELISA. (B) Jurkat cells were incubated PHA (1 μg/ml) alone or co-cultured with berberine (25 μg/ml) for 30 min. Total RNA was prepared and RT-PCR analysis was performed as described in materials and methods. Data were expressed as mean ± SEM. $^{+}$ $P < 0.01$ as indicate significant differences compared with vehicle group. ** $P < 0.01$ indicates significant differences compared with PHA alone group.

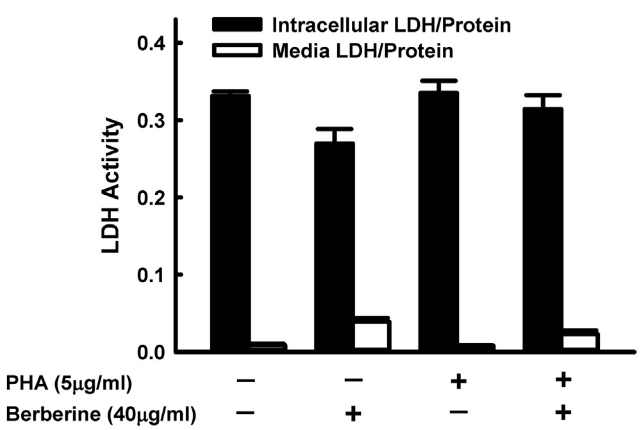

Fig. 3. Effects of berberine treatment on cytotoxicity of Jurkat cells. Jurkat cells (1×10^6 cells/ml) were pre-treated berberine (40 μg/ml) for 1 h and then stimulated with PHA (5 μg/ml) for 24 h. Collected cell lysis and media were stored in −80 °C and cytotoxicity was measured by LDH assay. Data were expressed as mean ± SEM.

PHA-evoked inflammatory responses were examined in cultured T-ALL Jurkat cells. Increased MAPK phosphorylation was observed in Jurkat cells treated with PHA (1 μg/ml) for 15 min (Fig. 4). In contrast, significantly attenuated phosphorylation of p38 was observed in berberine-treated (20 μg/ml) Jurkat cells with PHA (Fig. 4A and B). Berberine, however, did not affect the phosphorylation of ERK1/2 and JNK1/2 MAPK proteins (Fig. 4A, C and D). The above results indicated that berberine might inhibit the activation of phosphorylated p38, instead of ERK1/2 and JNK1/2 MAPK, when induced by PHA in Jurkat cells.

3.5. Effect of p38 inhibitors on IL-2 secretion from berberin-treated Jurkat cells

To confirm whether berberine exerted signaling pathways on lymphocytes *via* p38 MAPK lymphocytes, different concentrations of p38 inhibitors were applied in cell cultures. Significantly decreased IL-2 secretion was found in Jurkat cells with berberine and PHA. SB203580, Inhibitor of p38, recovered media IL-2 concentrations from Jurkat cells with PHA and berberine (Fig. 5). This result indicated that berberine appeared to regulate anti-inflammatory response by suppressing the p38 MAPK signaling pathway.

3.6. Effects of berberine on the expression of COX-2 protein in Jurkat cells with PHA

COX-2 is considered a pro-inflammatory enzyme that modulates the extent of inflammation status. Therefore, the effects of berberine on the inhibition of COX-2 expression were investigated. Treatment of Jurkat cells with PHA for 15 min led to a 1.3 fold increase in COX-2 expression. Interestingly, when the cells were incubated with berberine for 60 min before PHA stimulation, COX-2 expression was unexpectedly 1.7-fold increased compared with the vehicle group (Fig. 6). The above data indicated that pro-inflammatory enzyme, COX-2, was elevated by treatment of berberine in Jurkat cells.

3.7. Effect of berberine on cell cycle in Jurkat cells with PHA

We further analyzed the effect of berberine on cell cycle progression by flow cytometry in propidium iodide-stained cells for 1 h and then PHA for next 30 min. We observed that berberine-induced (20 μg/ml) cell cycle arrest of T-ALL Jurkat cells in the G0/G1 phase. As shown in Fig. 7, the percentage of cell populations between various phases of cell cycle in PHA-stimulated Jurkat cells was as follows: 38.38% in G0/G1, 27.93% in S and 33.69% in G2/M. Berberine (20 μg/ml) pre-treatment slightly enhanced G1 population to 40.72% and dropped the cell population in G2/M approximately to 2%, as compared with PHA-treated only group (Fig. 7B). This result demonstrated that berberine might arrest the growth of PHA-treated Jurkat cells.

4. Discussion

This study aimed to determine whether the preventive, anti-inflammatory responses of berberine in T-ALL Jurkat cells were regulated *via* the modulation of MAPK signaling pathway and cell cycle arrest. The results showed that 1) berberine inhibited IL-2 secretion from Jurkat cells treated with PHA, 2) attenuated IL-2 mRNA expression was observed in Jurkat cells treated with berberine and PHA, 3) berberine inhibited the expression of p-p38 proteins in Jurkat cells treated with PHA, 4) reduced IL-2 secretion from berberine-treated Jurkat cells could be recovered by adding p38 antagonist, 5) no cytotoxic effects of berberine on Jurkat cells were noticed at tested ranges up to 40 μg/ml, 6) berberine enhanced COX-2 protein expression in a dose-dependent

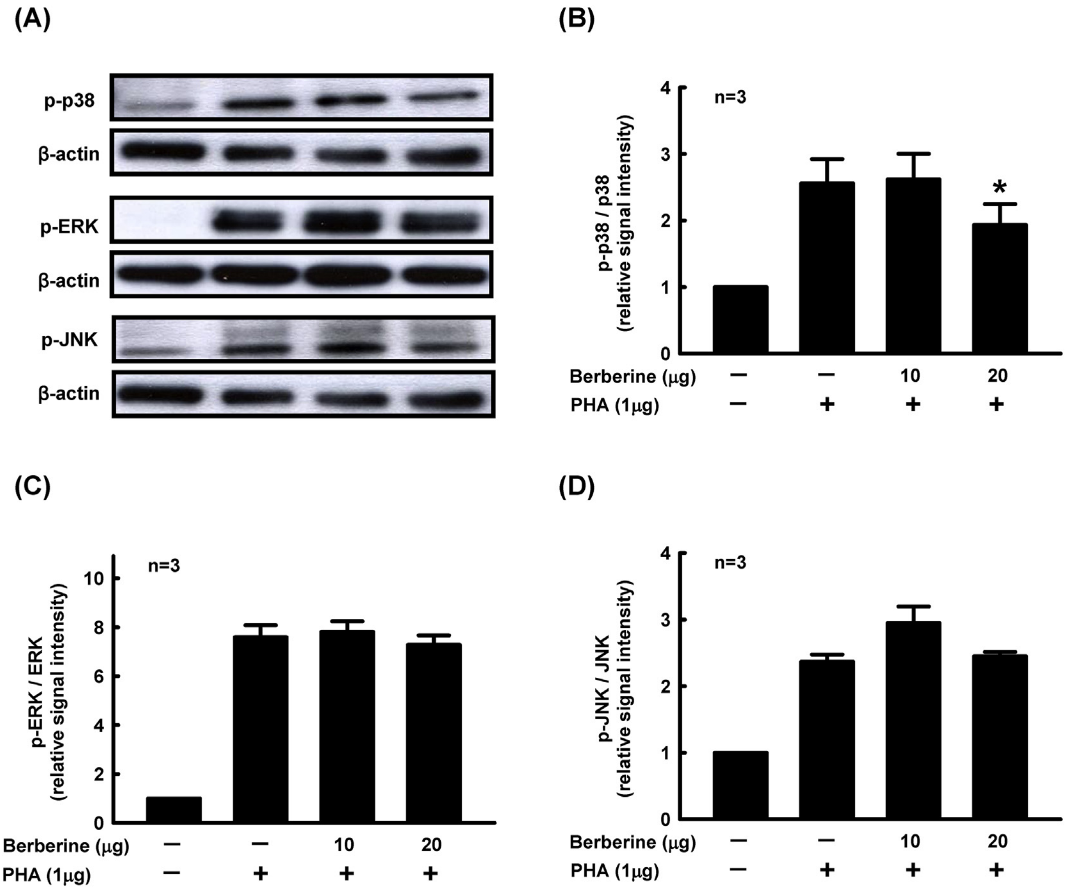

Fig. 4. Effect of berberine on PHA-evoked expression of phosphorylated MAPKs in Jurkat cells. Jurkat cells (1×10^6 cells/ml) were cultured in 10% FBS for 24 h. Different concentrations of berberine (10, 20 μg) were pre-treaded for 1 h and then exposed to PHA (1 μg/ml) for 15 min. Representative images are displayed in (A). The phosphorylated p38 (B), ERK (C), and JNK (D) MAPK proteins were analyzed by immunoblotting. Data were expressed as mean ± SEM. * $P < 0.05$ indicates significant differences compared with PHA alone group.

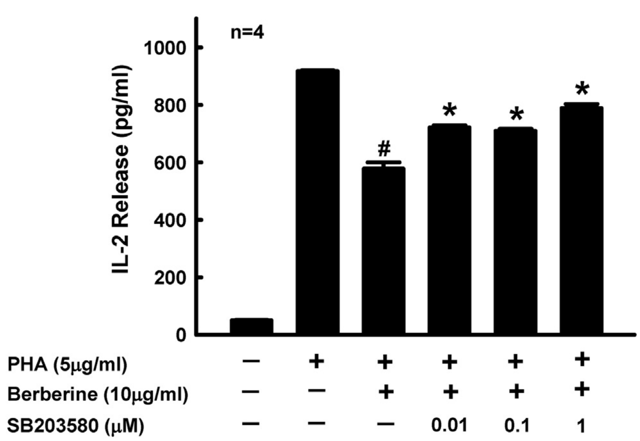

Fig. 5. p38 inhibitor SB203580 could reverse the mediation effect of berberine treatment on IL-2 secretion in PHA-stimulated Jurkat cells. Cells were cultured with SB203580 (0.01, 0.1 and 1 μM) for 1 h, then treated with PHA (5 μg/ml) for 24 h in the presence or absence of 10 μg/ml berberine. Culture media were harvested for measurement of IL-2 by ELISA. Data were expressed as mean ± SEM. # $P < 0.05$ as compared with PHA alone group. * $P < 0.05$ indicates significant differences compared with PHA + berberine group.

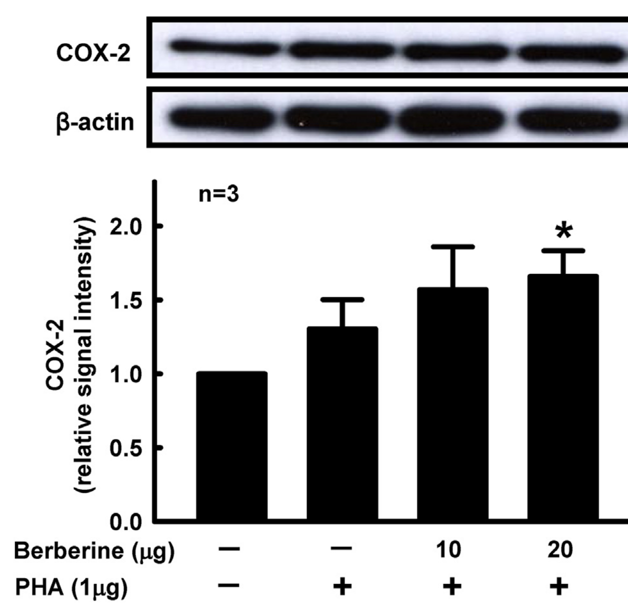

Fig. 6. Effect of berberine on PHA-induced COX-2 expression in Jurkat cells. Cells were pre-treated with 10 or 20 μg berberine for 1 h before the addition of 1 μg PHA for 15 min. Cell extracts were subjected to immunoblotting analysis with COX-2 antibodies. Data were expressed as mean ± SEM. * $P < 0.05$ indicates significant differences compared with PHA alone group.

manner, 7) berberine induced cell cycle arrest at G1 phase after PHA stimulation and decreased percentage of G2/M phase.

IL-2 is a cytokine which has been implicated to enhance T cell proliferation and differentiation *in vitro* [25]. Also, it is well known that IL-2 is mainly synthesized and secreted from activated T cells,

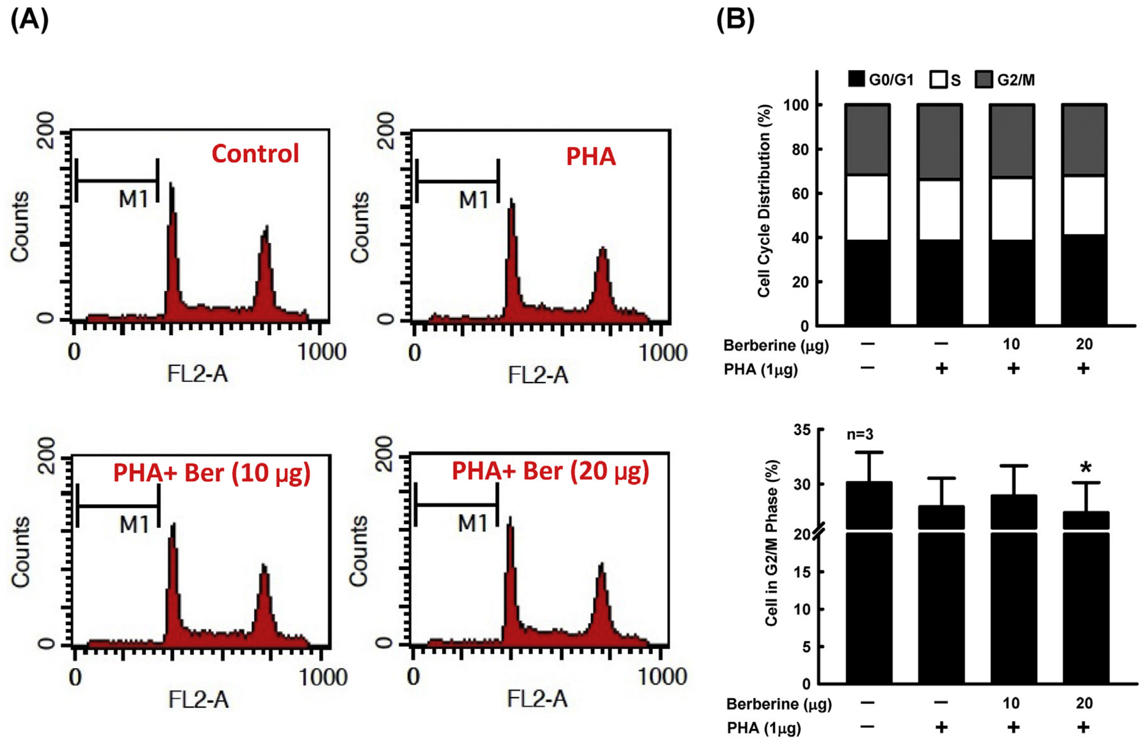

Fig. 7. Effect of berberine on induction of cell cycle arrest in Jurkat cells. Jurkat cells were treated with berberine (10 and 20 μg) for 1 h then exposed to PHA for 30 min. The cell cycle distribution was detected by flow cytometry analysis. (A) Representative flow histograms showing the cell cycle profiling of Jurkat cells treated with PHA and berberine at the indicated concentration. (B) Analysis of G1, S, and G2/M phases (upper panel) and quantization of the cells in G2/M phase (lower panel) of cell cycle in untreated and treated cells with PHA and berberine. * $P < 0.05$ indicates significant differences compared with PHA alone group.

especially CD4+ T-helper cells [26]. When antigen-containing pathogens attach to T cells, IL-2 plays an important role in generation of memory T cells [27]. In contrast, IL-2 possesses the immune-suppressive functions, as exerted by IL-2, including promotion of activation-induced CD8 + -T cell death [28] and downregulation of T cell number after an immune response [29]. Also, IL-2 exerts its anti-inflammatory effect, like other proinflammatory cytokine IFN-γ [30], by enhancing the generation of regulatory T cells [31] and suppressing T-helper 17 cells [32]. Our study showed that berberine inhibited IL-2 secretion and IL-2 mRNA expression in PHA-treated lymphocytic Jurkat cells. This result indicated berberine could modulate, at least, the immune function.

As a member of the MAPK family, p38 and JNK are typical signaling pathways in regulating IL-2 secretion in T lymphocyte [33]. Berberine, the major alkaloidal component of *Rhizoma coptidis* (Huanglian), might contribute to anti-inflammatory activity through inhibition of MAPK signaling pathway. Previous studies showed that berberine inhibits the inflammatory responses in carrageenan-induced paw edema mice and in macrophage activated by LPS *via* downregulation of p38 and JNK expression [34,35]. Thus, p38 and JNK signaling have been viewed as the target proteins for the anti-inflammatory action by berberine. Our results showed that berberine significantly regulated PHA-induced phosphorylation of p38 only, but affected neither p-JNK nor p-ERK, suggesting that p38 had a crucial role in berberine-mediated IL-2 secretion in Jurkat cells. In turn, berberine might exert potential anti-inflammatory effects by attenuating p38 MAPK signaling expression.

There are two types of cyclooxygenase (COX), COX-1 and COX-2. COX-1 is mainly constitutively expressed in different cell types throughout human body. COX-2, expressed in response to different stimuli (*e.g.* cytokines, growth factors, or mitogens), is an inducible form [36]. Also, studies found that COX-2 often overexpress in malignant tumor, including breast, bladder, and colon cancer [37]. Berberine attenuates the expression of COX-2 mRNA in impaired hippocampus induced by administration of scopolamine in rats [38]. In rats' small intestinal mucosa impaired by LPS-induced acute endotoxemia, increased COX-2 expression can be inhibited by berberine [39]. Through decreased expression of COX-2, berberine curtails melanoma cell migration and metastasis [40]. In oral cancer cells, apoptosis induced by berberine could be in a COX-2-dependent manner [17]. Moreover, berberine exerts its inhibitory effects on COX-2 transcriptional activity in colon cancer cells in a dose- and time-dependent manner [41]. Pandey et al. found that berberine displaying the anti-inflammatory activity in Jurkat cells may be mediated through the inhibition of COX-2 expression [42]. In addition, berberine inhibits the LPS-induced COX-2 expression in peritoneal macrophages and RAW 264-7 cells [35]. In contrast to these studies, Kim et al. showed that berberine does not affect the LPS-induced COX-2 gene expression in RAW264.7 macrophages [43]. Opposite to above inhibitory effects of berberine on cancer cells, our results in this study showed that berberine stimulated the expression of COX-2 protein in Jurkat cells. More studies are still required to reveal the relation between berberine on IL-2 secretion and COX-2 expression.

It is well documented that cell cycle control is crucial in cellular division. Uncontrolled cell proliferation has been proven to be associated with inflammatory processes. In contrast, arrest proliferation of Jurkat cells is suggested to be an effective process to eliminate and abrogate inflammatory responses [44]. Berberine causing attenuation of cytokine secretion in human multiple myeloma cells could be attributed to apoptosis or cell cycle arrest at the G2/M phase [45]. Based on previous studies, we hypothesized that berberine might be an effective compound working against inflammatory responses through the regulation of cell cycle and cytokine production. We discovered that berberine efficiently attenuated cell growth by inducing G0/G1 cell cycle arrest in T-ALL Jurkat cells. This phenomenon is possible to be the mechanism for the anti-cancer and anti-inflammation properties regulated by berberine, which suggests our finding thus extending earlier observations by demonstrating that the action of berberine on cell cycle might be applied for anti-inflammation and cancer therapy.

In conclusion, we revealed that berberine could diminish PHA-evoked IL-2 secretion, potentially *via* the attenuation of phosphorylation of p38 MAPK expressions, as well as arrest cell cycle in Jurkat cell. Our observation indicated that berberine could be a potential therapeutic candidate for the treatment of inflammatory diseases and leukemia patients in the future.

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