Baicalein inhibits TNF-α-induced NF-κB activation and expression of NF-κB-regulated target gene products

JUNBO LI, JUAN MA, KE SI WANG, CHUNLIU MI, ZHE WANG, LIAN XUN PIAO, GUANG HUA XU, XUEZHENG LI, JUNG JOON LEE and XUEJUN JIN

Key Laboratory of Natural Resources of Changbai Mountain and Functional Molecules, Ministry of Education, Molecular Cancer Research Center, College of Pharmacy, Yanbian University, Yanji, Jilin 133002, P.R. China

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Abstract. The nuclear factor-κB (NF-κB) transcription factors control many physiological processes including inflammation, immunity, apoptosis and angiogenesis. In our search for NF-κB inhibitors from natural resources, we identified baicalein from *Scutellaria baicalensis* as an inhibitor of NF-κB activation. As examined by the NF-κB luciferase reporter assay, we found that baicalein suppressed TNF-α-induced NF-κB activation in a dose-dependent manner. It also inhibited TNF-α-induced nuclear translocation of p65 through inhibition of phosphorylation and degradation of IκBα. Furthermore, baicalein blocked the TNF-α-induced expression of NF-κB target genes involved in anti-apoptosis (cIAP-1, cIAP-2, FLIP and BCL-2), proliferation (COX-2, cyclin D1 and c-Myc), invasion (MMP-9), angiogenesis (VEGF) and major inflammatory cytokines (IL-8 and MCP1). The flow cytometric analysis indicated that baicalein potentiated TNF-α-induced apoptosis and induced G1 phase arrest in HeLa cells. Moreover, baicalein significantly blocked activation of p38, extracellular signal-regulated kinase 1/2 (ERK1/2). Our results imply that baicalein could be a lead compound for the modulation of inflammatory diseases as well as certain cancers in which inhibition of NF-κB activity may be desirable.

Correspondence to: Professor Xuejun Jin, Key Laboratory of Natural Resources of Changbai Mountain and Functional Molecules, Ministry of Education, Molecular Cancer Research Center, College of Pharmacy, Yanbian University, Yanji, Jilin 133002, P.R. China
E-mail: xjjin@ybu.edu.cn

Abbreviations: NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; IκBα, inhibitor of NF-κB alpha; IL-8, interleukin 8; cIAP1, cellular inhibitor of apoptosis protein 1; cIAP2, cellular inhibitor of apoptosis protein 2; FLIP, cellular FLICE inhibitory protein; BCL-2, B-cell lymphoma-2; MCP1, monocyte chemotactic protein 1; COX-2, cyclooxygenase-2; MMP-9, matrix metalloproteinase-9; VEGF, vascular endothelial growth factor; c-Myc, cellular-myelocytomatosis viral oncogene

Key words: baicalein, nuclear factor-κB, IκBα, inflammation, cancer

Introduction

The transcription factor NF-κB was discovered in 1986 as a nuclear factor that binds to the enhancer element of the immunoglobulin kappa light-chain of activated B cells (thereby coining the abbreviation NF-κB) (1). NF-κB represents a family of eukaryotic transcription factors participating in the regulation of various cellular genes involved in the immediate early processes of immune, acute phase and inflammatory responses as well as genes involved in cell survival (2). A commonly known NF-κB consists of a RelA (p65)/p50 heterodimer and RelA (p65) contains a C-terminal transactivation domain in addition to the N-terminal Rel-homology domain, thus, serving as a critical transactivation subunit of NF-κB (3). In the resting state, the inactive NF-κB is retained in the cytoplasm by an inhibitory subunit called IκB. The phosphorylation of IκB by the IκB-kinase (IKK) containing IKKα, IKKβ and the regulatory protein NF-κB essential modifier (NEMO) is a key step in NF-κB activation in response to various stimuli such as tumor necrosis factor-α (TNF-α) (3,4). In response to stimulation, IκBs are rapidly ubiquitinated and degraded by 26S proteasome complex and the release of IκB unmasks the nuclear localization signal and results in the translocation of NF-κB to the nucleus where it can bind to κB sites, followed by the activation of specific target genes (5).

It is reported that NF-κB regulates several hundreds of genes, including those involved in immunity and inflammation, anti-apoptosis, cell proliferation, tumorigenesis and the negative feedback of the NF-κB signal (6). NF-κB regulates major inflammatory cytokines, including interleukin 8 (IL-8), monocyte chemotactic protein 1 (MCP1), many of which are potent activators for NF-κB. NF-κB has been shown to regulate the expression of several genes whose products are involved in tumorigenesis (2), including cyclooxygenase-2 (COX-2), cyclin D1, c-Myc, apoptosis suppressor proteins such as cellular inhibitor of apoptosis 1 (cIAP-1), cellular inhibitor of apoptosis 2 (cIAP-2), cellular FLICE inhibitory protein (FLIP), B-cell lymphoma-2 (BCL-2) and genes required for invasion and angiogenesis such as matrix metalloproteinase (MMP-9) and vascular endothelial growth factor (VEGF).

Baicalein is a naturally occurring flavonoid which is an active component of *Scutellaria baicalensis* (7). *Scutellaria baicalensis* is one of the most popular traditional Chinese medicine herbal remedies used in China and several oriental countries for treatment of inflammation, bacterial and viral infections, and have been shown to possess anticancer activities *in vitro* and *in vivo* in mouse tumor models (8). Previous investigations also showed that baicalein have multiple pharmacological activities including anti-oxidant effects, chemo-preventive effects against several types of cancer and anti-inflammatory effect (9-11). However, the molecular mechanism of anti-inflammatory and anticancer effects has not been sufficiently explained. In the present study, whether baicalein exerts its anti-inflammatory and anticancer effects through suppression of the NF-κB pathway was investigated. Our data demonstrated baicalein downregulates the expression of target genes involved in antiapoptosis (cIAP-1, cIAP-2, BCL-2 and FLIP), proliferation (cyclin D1, COX-2 and c-Myc), invasion (MMP-9), angiogenesis (VEGF) and major inflammatory cytokines (IL-8 and MCP1). Taken together, these findings support further studies of baicalein as candidate for treatment of inflammation and cancer.

Materials and methods

Cell culture and reagents. HeLa cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37˚C with 5% CO_2 atmosphere in a humidified incubator. TNF-α was obtained from R&D Systems (Minneapolis, MN, USA). Baicalein was from Sigma-Aldrich (St. Louis, MO, USA) and its structure is shown in Fig. 1A. The purity of baicalein was over 99% in HPLC analysis.

MTT assay. HeLa cells were seeded in 96-well plates at a density of $1x10^5$ cells/ml and cultured overnight. Following cell treatment with different concentrations of baicalein (10-100 μM) for 12 h, 10 μl MTT solution (5 mg/ml) was added into each well and incubated with cells for 4 h at 37˚C. Then, DMSO was added to dissolve the formazan crystals. The absorbance at 570 nm was measured by Multiskan GO.

Plasmids, transfections and luciferase reporter assay. A pNF-κB-Luc plasmid for NF-κB luciferase reporter assay was obtained from Strategene (La Jolla, CA, USA). Transfections were performed as previously described (12). NF-κB-dependent luciferase activity was measured using the Dual-luciferase reporter assay system. Briefly, HeLa cells ($1x10^5$ cells/well) were seeded in a 96-well plate for 24 h. The cells were then transfected with plasmids for each well and then incubated for a transfection period of 24 h. After that, the cell culture medium was removed and replaced with fresh medium containing various concentrations of baicalein for 6 h, followed by treatment with 10 ng/ml of TNF-α for 6 h. Luciferase activity was determined in MicroLumat plus luminometer (EG&G Berthold, Bad Wildbad, Germany) by injecting 100 μl of assay buffer containing luciferin and measuring light emission for 10 sec. Co-transfection with pRL-CMV (Promega, Madison, WI, USA), which expresses *Renilla* luciferase, was performed to enable normalization of data for transfection efficiency.

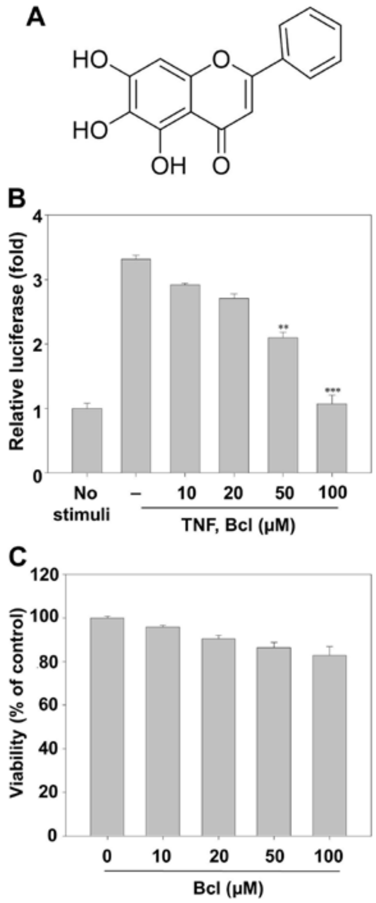

Figure 1. Effect of baicalein (Bcl) on the TNF-α-induced NF-κB-dependent reporter gene expression. (A) Structure of baicalein (Bcl). (B) HeLa cells were transiently transfected with an NF-κB-dependent reporter gene for 24 h and then pretreated for 6 h with the indicated concentrations of baicalein (Bcl) followed by stimulation for 6 h with TNF-α (10 ng/ml), and the luciferase activity was determined as described in Materials and methods. Data are presented as mean ± standard deviation of three independent experiments. **P<0.01, ***P<0.001, significantly different when compared with TNF-α-stimulated normal cells. (C) HeLa cells were treated with the indicated concentrations of baicalein (Bcl). After 12-h incubation, cell viability was determined by MTT assays. Data are presented as mean ± standard deviation of three independent experiments.

Western blot analysis. HeLa cells were cultured in 10 cm-dishes and allowed to adhere for 24 h. After treatment with various concentrations of baicalein in the presence or absence of TNF-α (10 ng/ml), then, cells were harvested and lysed. An equal amount of protein was separated by SDS-polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membrane was blocked with 5% non-fat dried milk for 1 h, the membrane was incubated with the primary antibodies. Antibodies for IκBα, phosphor (Ser32)-specific IκBα, p65, PARP, caspase-8, cIAP-1, cIAP-2, phospho-ERK, phospho-JNK, phospho-p38, ERK, JNK and P38 were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies for COX-2, MMP-9, VEGF, BCL-2, FLIP, and Topo-I were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody for α-tubulin was from Sigma-Aldrich. After binding of an appropriate secondary antibody coupled to horseradish peroxidase. Then the immunoreactive bands were visualized by enhanced chemiluminescence according to the manufacturer's instructions (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Immunofluorescence of NF-κB p65. HeLa cells were seeded into 24-well plates at $1x10^4$ cells/well. Twenty-four hours later, cells were pretreated with baicalein (100 μM) for 12 h, followed by treatment with TNF-α (10 ng/ml) for 30 min. Cells pretreated with DMSO and TNF-α (10 ng/ml) alone were used as negative and positive controls, respectively. Subsequently, the cells were washed in PBS, fixed at room temperature with 4% paraformaldehyde, and permeabilized with 0.2% Triton X-100. Immunofluorescence staining was performed according to the standard procedures. Briefly, the treated cells were first stained with the anti-p65 antibody followed by incubation with FITC conjugated anti-rabbit IgG secondary antibody and nuclei were counterstained with DAPI. The staining was examined using a fluorescence microscope.

Apoptosis assays. Apoptosis assays were performed as previously described (13). Annexin V-staining was performed using Annexin V-FITC apoptosis detection kit (BD Biosciences, San Jose, CA, USA) following the instructions of the manufacturer. Briefly, after incubation, detached cells were collected with the supernatant, pelleted by centrifugation. The adherent cells were rinsed twice with medium before harvesting. Then cells were harvested in trypsin without EDTA. Detached and adherent cells were finally pooled and were resuspended in binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) to a final concentration of $1x10^5$ cells/ml. The pooled cells were stained with Annexin V-FITC and 2 μg/ml propidium iodide for 15 min at 37˚C in the dark. To the samples was added 400 μl binding buffer before analyzed by flow cytometry. The CellQuest software was used to analyze the data (Becton-Dickinson, Franklin Lakes, NJ, USA).

RT-PCR analysis. Reverse transcription-PCR (RT-PCR) was performed to determine NF-κB target gene expression as previously described (14). In brief, HeLa cells were preincubated with the indicated concentrations of baicalein at 37˚C for 12 h and then followed by treatment with 10 ng/ml of TNF-α for 12 h. Cells were harvested and washed twice with ice-cold PBS, and then total RNA was isolated from cells using RNeasy Mini kits according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). Complementary DNA was synthesized from 1 μg of total RNA in a 20 μl reverse transcription reaction mixture according to the manufacturer's protocol (Takara Bio, Kyoto, Japan). The PCR primers for interleukin-8 (IL-8), 5'-TCTGCAGCTCTGTGTGAAGG-3' and 5'-ACTTCTCC ACAACCCTCTG-3'; for MCP1, 5'-CCCCAGTCACCTGC TGTTAT-3' and 5'-AGATCTCCTTGGCCACAATG-3'; for c-Myc, 5'-CTCTCAACGACAGCAGCCCG-3' and 5'-CCA GTCTCAGACCTAGTGGA-3'; for GAPDH, 5'-ACCAGG TGGTCTCCTCT-3' and 5'-TGCTGTAGCCAAATTCG TTG-3'. The mRNA levels of all genes were normalized to that of GAPDH. PCR products were separated on 3% agarose gel and then stained with ethidium bromide. Stained bands were visualized under UV light and photographed.

Cell cycle assay. HeLa cells were cultured in 6-well plates until 70-80% confluent. The cells were then treated with baicalein at indicated concentrations in serum-free medium. Cells were then washed with PBS, fixed in ice-cold 70% ethanol and stained with PI buffer (0.1% Triton X-100, 0.2 mg/ml RNaseA, and 0.05 mg/ml PI) for 30 min. The DNA content was measured using a FACSCalibur flow cytometer with CellQuest software (Becton-Dickinson). For all assays, 10,000 events were counted. The ModFit LT v4.0 software package (Verity Software House, Inc., Topsham, ME, USA) was used to analyze the data.

Statistical analysis. All values are expressed as mean ± SD. A comparison of the results was performed with one-way ANOVA and Tukey's multiple comparison tests (GraphPad Software, Inc., San Diego, CA, USA) and the Student's t-test. P-values of <0.05 were considered statistically significant.

Results

Baicalein inhibits TNF-α-induced NF-κB activation. We first investigated the effect of baicalein on TNF-α-induced NF-κB activation by NF-κB-dependent reporter gene assay. HeLa cells were transiently transfected with the NF-κB-regulated luciferase reporter vector. When the HeLa cells were pretreated with various concentration of baicalein, TNF-α-induced NF-κB-reporter gene expression was inhibited in a dose-dependent manner (Fig. 1B). We evaluated the cytotoxic effects of baicalein on HeLa cell survival by MTT assay. The results showed that up to 100 μM of baicalein had no cellular toxicity on HeLa cells (Fig. 1C).

Baicalein inhibits TNF-α-induced IκBα phosphorylation and degradation, and p65 nuclear translocation. Transcriptional activity of NF-κB is dependent on IκBα phosphorylation. To determine whether baicalein inhibition of TNF-α-induced NF-κB activation, total cell extracts were prepared with baicalein and then exposed to TNF-α for various time periods, phosphorylation and degradation of IκBα was analyzed by western blot analysis. The results showed that baicalein potently inhibited the TNF-α-induced phosphorylation and degradation of IκBα in a dose-dependent manner (Fig. 2A). In addition, TNF-α-induced phosphorylation and degradation of IκBα were occurred as quickly as 15 min (Fig. 2B). Next, we examined whether baicalein modulates TNF-α-induced nuclear translocation of p65. Nuclear extracts were pretreated with baicalein and then exposed to TNF-α for various time periods and analyzed p65 nuclear translocation by western blot analysis. The results showed that baicalein also potently inhibited TNF-α-induced nuclear translocation of p65 in a dose-dependent manner (Fig. 2C), and the earliest inhibition also occurred within 15 min after TNF-α addition (Fig. 2D). To further confirm these results, the immunofluorescence staining assay was performed. Immunofluorescence images showed that in untreated, p65 was localized in the cytoplasm. In TNF-α alone treated, p65 was translocated to the nucleus. Followed by inhibited nuclear translocation of p65 with baicalein pretreatment (Fig. 2E).

Baicalein inhibits TNF-α-induced NF-κB-regulated gene products. NF-κB regulates the expression of anti-apoptotic gene products cIAP-1, cIAP-2, BCL-2 and FLIP, prolif-

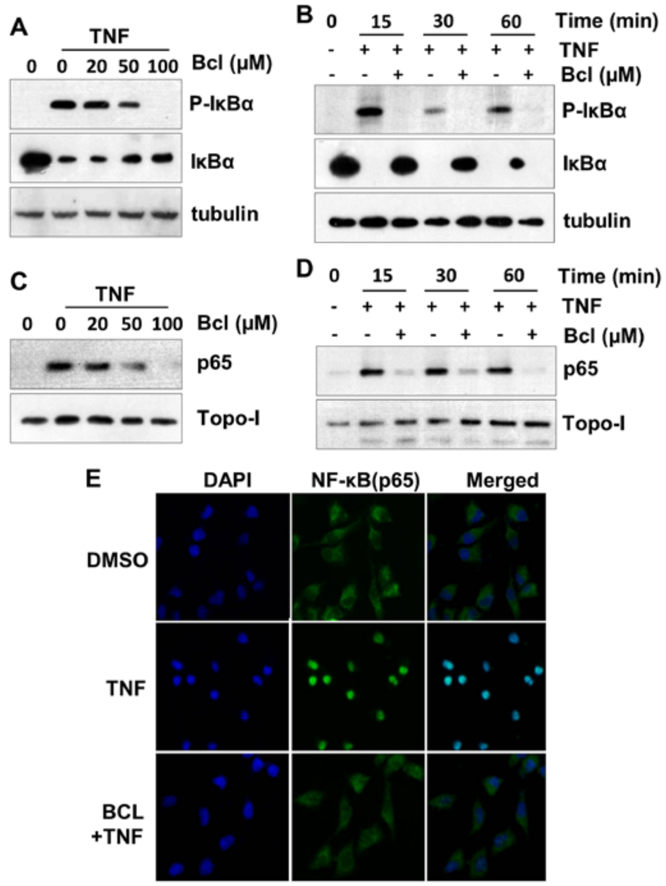

Figure 2. Effect of baicalein (Bcl) on the TNF-α-induced phosphorylation and degradation of IκBα and p65 nuclear translocation. (A) HeLa cells were preincubated with indicated concentrations of baicalein (Bcl) for 12 h and then treated with TNF-α (10 ng/ml) for 30 min. Cytoplasmic extracts were analyzed by western blot analysis using indicated antibodies for p-IκBα, IκBα and tubulin. (B) HeLa cells were incubated with 100 μM baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for the indicated times. Cytoplasmic extracts were analyzed by western blot analysis using indicated antibodies for p-IκBα, IκBα and tubulin. (C) HeLa cells were preincubated with indicated concentrations of baicalein (Bcl) for 12 h and then treated with TNF-α (10 ng/ml) for 30 min. Nuclear extracts were analyzed by western blot analysis using indicated antibodies for p65 and Topo-I. (D) HeLa cells were incubated with 100 μM baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for the indicated times. Cells were harvested at the indicated time-points and then nuclear extracts were prepared. Nuclear p65 was detected by western blot analysis. (E) HeLa cells were incubated with 100 μM baicalein (Bcl) for 12 h and followed by TNF-α (10 ng/ml) stimulation for 30 min. After fixation, cells were stained with specific anti-p65 antibody followed by Alex Flour® 488 (green), and the nucleus was counterstained with DAPI (blue) and examined by fluorescence microscopy. Scale bars, 20 μm. Images were acquired for each fluorescence channel, using suitable filters with x40 objective. The green and blue images were merged using ImageJ software.

eration gene products COX-2 and cyclin D1, invasion and angiogenesis gene products MMP-9 and VEGF, which are known to be induced by TNF-α. We used western blotting to determine whether baicalein inhibits the expression of these gene products in HeLa cells. Our results showed that baicalein markedly downregulated TNF-α-induced expression of all these proteins in a dose-dependent manner (Fig. 3A). NF-κB regulates major inflammatory cytokines and proliferation, including IL-8, MCP1 and c-Myc. Thus, we also investigated whether baicalein can modulate TNF-α-induced expression of these genes by RT-PCR analysis. The results showed that

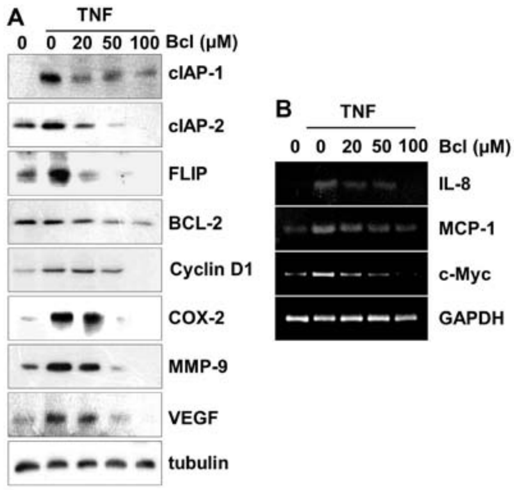

Figure 3. Baicalein (Bcl) inhibits TNF-α-induced NF-κB-regulated gene products. (A) HeLa cells were incubated with indicated concentrations of baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for 12 h. Whole cell extracts were analyzed by western blot analysis using indicated antibodies for cIAP-1, cIAP-2, FLIP, BCL-2, COX-2, cyclin D1, MMP-9, VEGF and tubulin. (B) HeLa cells were incubated with indicated concentrations of baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for 12 h. Total RNA was isolated from cells, reverse-transcribed and analyzed by RT-PCR assay as described in Materials and methods. GAPDH was used to show equal loading of total RNA.

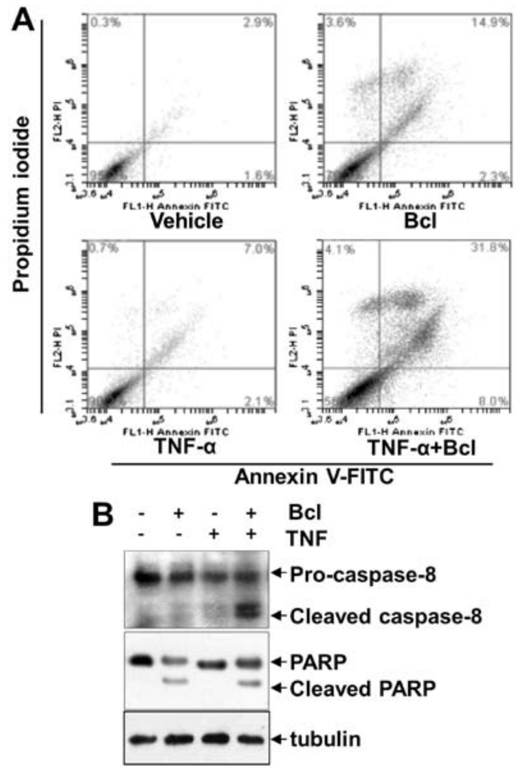

Figure 4. Effect of baicalein (Bcl) on the TNF-α-induced apoptosis. (A) HeLa cells were pretreated with 100 μM baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for 12 h, and subsequently stained with Annexin V-FITC and propidium iodide, followed by analysis using a flow cytometer. Representative plots of one set of triplicate experiments. Early apoptotic cells (Annexin-V$^+$ and PI) are displayed in the lower right quadrant and late apoptotic cells (Annexin-V$^+$ and PI$^+$) are shown in the upper right quadrant. (B) HeLa cells were pretreated with 100 μM baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for 12 h. Whole cell extracts were analyzed by western blot analysis using indicated antibodies for cleaved capase-8, cleaved PARP and tubulin.

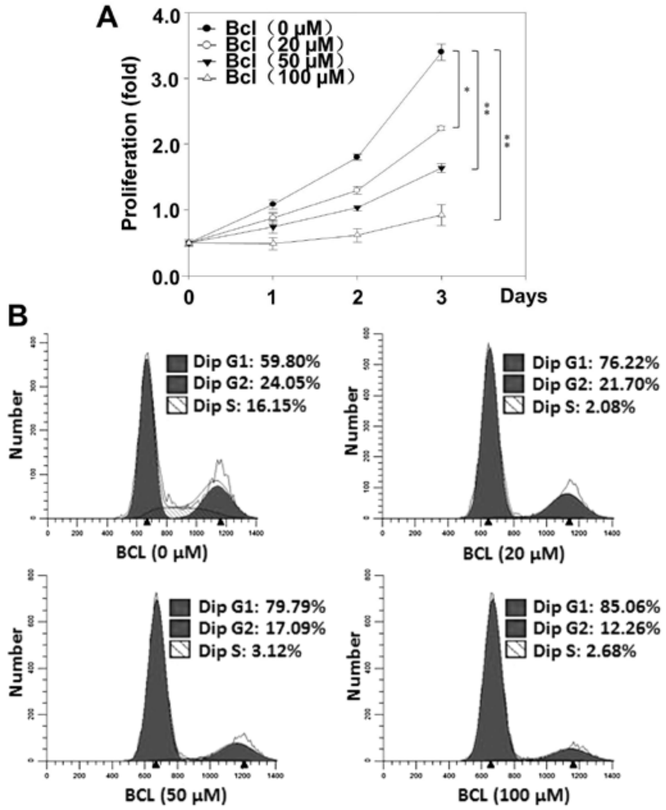

Figure 5. Effect of baicalein (Bcl) on the TNF-α-induced tumor cell proliferation. (A) HeLa cells were plated in triplicate, treated with 0, 20, 50 or 100 μM baicalein (Bcl), and subjected to MTT assay on days 1, 2 and 3 to analyze cell proliferation. Absorbance was measured at 570 nm. $^{*}P<0.05$, $^{**}P<0.01$, significant with respect to the control. (B) HeLa cells were treated with baicalein (Bcl) at the indicated concentrations for 24 h. The DNA content of propidium iodide-stained nuclei was analyzed by FACSCalibur flow cytometry, as described in Materials and methods.

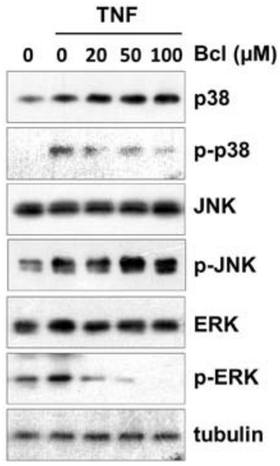

Figure 6. Effect of baicalein (Bcl) on the TNF-α-induced activation of MAP kinases. HeLa cells were incubated with the indicated concentrations of baicalein (Bcl) for 12 h and then incubated with TNF-α (10 ng/ml) for 30 min. Total extracts were analyzed by western blot analysis using the indicated antibodies for ERK, phospho-ERK, JNK, phospho-JNK, p38 and phospho-p38. Tubulin level was used as a loading control.

baicalein blocked TNF-α-induced mRNA expression of IL-8, MCP1 and c-Myc in a dose-dependent manner (Fig. 3B).

Baicalein potentiates TNF-α-induced apoptosis. HeLa cells were sequentially treated with baicalein and TNF-α, then stained with Annexin V-FITC and propidium iodide and analyzed using a flow cytometer. As shown in Fig. 4A, treatment of HeLa cells with vehicle only, TNF-α alone, and baicalein alone induced apoptosis of 4.5, 9.1 and 17.2% respectively. However, combined treatment of the cells with TNF-α and baicalein resulted in a significant potentiated apoptosis of HeLa cells (39.8%). To assess whether baicalein can enhance the TNF-α-induced apoptosis, the activation of caspases-8 and PARP was also investigated. Our results showed that baicalein alone had little effect on caspases-8 and PARP cleavage, However, combined treatment of TNF-α with baicalein potentiated their activation (Fig. 4B). These results together indicate that baicalein enhances the apoptotic effects of HeLa cells by TNF-α.

Baicalein inhibits the proliferation of HeLa cells via blocking cell cycle progression in the G1 phase. Next, in order to investigate the effects of baicalein on HeLa cell proliferation, the proliferation assay were performed. Indeed, as in MTT experiments, the strongest growth inhibitory effect was observed at 72 h of baicalein (100 μM) incubation (Fig. 5A). In order to elucidate if impairment of cell cycle participate in the reduction of the HeLa cell growth rate induced by baicalein, the flow cytometric analyses of cell cycle were performed. Our results showed that baicalein increased the population of G1 phase cells. These results suggest that baicalein inhibits cell proliferation through blocking cell cycle progression in G1 phase in HeLa cells.

Baicalein inhibits TNF-α-induced phosphorylation of ERK1/2 and p38. The inflammatory response can be activated through the MAP kinase pathway. Thus, we determined whether baicalein can inhibition TNF-α-induced inflammatory responses through MAPK signaling. Since the MAPK pathway is phosphorylation-dependent, the phosphorylated proteins were easily detectable by western blot analysis. The results showed that baicalein decreased TNF-α-induced ERK1/2 and p38 by inhibiting their phosphorylation (Fig. 6).

Discussion

NF-κB is normally retained in the cytoplasm through interaction with its inhibitor IκB. IκB exerts its inhibitory effects by associating with the Rel homology domain of NF-κB proteins, effectively masking their nuclear localization signals (15-17). Our results determined that baicalein suppresses TNF-α-induced NF-κB activation through the inhibition of IκB phosphorylation and degradation, p65 nuclear translocation. Our studies also determined that baicalein inhibits TNF-α-induced NF-κB-regulated target gene products that are associated with inflammation, apoptosis, tumor cell proliferation, cell cycle, invasion and angiogenesis.

Apoptosis is an important mechanism to eliminate unwanted cells, and deregulation of this process is implicated in the pathogenesis of cancer development (18). Our results showed that baicalein inhibits TNF-α-induced expression of antiapoptotic proteins such as cIAP-1, cIAP-2, FLIP and BCL-2, which are known to be regulated by NF-κB. Furthermore, the flow cytometric analysis showed that baicalein enhanced TNF-α-induced apoptosis. The loss of caspase activation appears to be central to the prevention of most cell death events in cancer. Finding strategies to overcome caspase inhibition will be valuable for the development of novel cancer treatments (19). We also found that baicalein potentiated TNF-α-induced activition of caspases-8 and PARP, which suggested that baicalein enhances cell apoptosis signaling by TNF-α. Moreover, our results demonstrated that baicalein suppressed TNF-α-induced expression of MMP-9, VEGF and COX-2, which are major mediators involved in tumor invasion, metastasis and proliferation (20-22). Flow cytometric analysis with PI staining indicated that baicalein can suppress cell proliferation via blocking cell cycle progression in the G1 phase. Cyclin D1 is a protein that is expressed relatively early in the cell cycle and is crucial for control of G1 phase (8). We also observed baicalein suppressed TNF-α-induced expression of cyclin D1 protein in HeLa cells.

MAP kinases are another signaling pathway that plays a critical role in inflammation through activation of NF-κB (23). This kinase family is composed of several subgroups, such as ERK, JNK and p38. Therefore, experiments were performed to determine whether baicalein regulates TNF-α-stimulated expression of MAP kinases in HeLa cells. Our results showed that baicalein prevented the activation of p38 and ERK1/2. The anti-inflammatory effects of baicalein have been determined via investigation of several major inflammatory cytokines, such as IL-8 and MCP1, which are regulated by NF-κB and are also potent activators for NF-κB. NF-κB-binding sites have been identified in the promoter of over 300 different genes, and these genes are known to regulate a wide variety of cellular responses affected by baicalein. Overall, our results provide the molecular basis through which baicalein mediates its anti-inflammatory and anticancer effects. We conclude that baicalein is a potent inhibitor of NF-κB and NF-κB-regulated gene products, and may be a valuable new drug candidate for the treatment of inflammation and cancer.

Acknowledgements

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