Berberine Inhibits Human Neuroblastoma Cell Growth through Induction of p53-dependent Apoptosis

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Abstract. *Berberine, an alkaloid, has anti-tumor properties in some cancer cells, but action mechanisms are not clear yet. We here investigated the anticancer activity of berberine and possible mechanisms in human neuroblastoma SK-N-SH and SK-N-MC cells. The p53-expressing cells, SK-N-SH (IC_{50}=37 μM) were more susceptible to berberine than the p53-deficient cells, SK-N-MC (IC_{50}≥100 μM) without cytotoxic effect on the cortical neuronal cells. Berberine caused cell cycle arrest in G_0/G_1 phase and apoptotic cell death, and these effects were much greater in SK-N-SH cells than those in SK-N-MC cells. Berberine much greatly decreased G_0/G_1 phase-associated cyclin and cyclin-dependent kinase (cyclin D1, cyclin E, Cdk2, and Cdk4) expression, and increased apoptotic gene expression and activation of caspase-3 in SK-N-SH cells. Exploration of p53 siRNA or pifithrin-α (PFT-α), a p53 inhibitor, in the SK-N-SH cells resulted in increase of IC_{50} values for cell viability, and decreased apoptotic cell death, expression of p53 and activation of caspase-3. Therefore, these results showed that berberine causes p53-dependent apoptotic death of neuroblastoma cells, and suggested that berberine may be useful as an anticancer agent for neuroblastoma.*

Berberine is an isoquinoline alkaloid that has been isolated from *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). Berberine possesses a wide range of biochemical and pharmacological activities such as anti-diarrheal (1), anti-arrhythmic (2), and anti-inflammatory (3) activities. Berberine has been examined for anticancer activity in several types of cancer cells (4-13). Several possible mechanisms of anticancer effects of berberine have been also suggested. Berberine inhibits human colon cancer cell growth effectively by inhibiting cyclooxygenase-2 transcriptional activity and arylamine *N*-acetyltransferase activity (14, 15). Berberine also inhibits DNA topoisomerase I and II in biochemical systems (7). Because berberine interacts with DNA, it is not surprising that berberine could act anticancer drug as an intercalating agent (7, 8). Activation of caspase-3 as a possible anticancer mechanism of bereberine was also suggested in many types of cancer cells (8-13). However, the anticancer effect of berberine on neuroblastoma and possible mechanisms of anticancer activity have not been studied.

Being a key partner in the cellular response to stress, p53, a tumor suppressor protein, serves as the major obstruction for carcinogenesis. Indeed, approximately 50% of human cancers bear *p53* gene mutations (16). p53 is activated as a transcription factor in response to *e.g.* oncogene activation, hypoxia and DNA damage, resulting in growth arrest and/or apoptosis by stimulating the expression of various p53 target genes such as $p21^{Waf1/Cip1}$, *Bax, Puma, Noxa, Apaf-1, Fas,* and *DR5* (17-18). p53 can also transcriptionally repress the expression of anti-apoptotic proteins, *e.g.* Bcl-2, Bcl-XL or survivin (19). *Bax* gene product dimerizes with Bcl-2 and prevents the activity of Bcl-2 from blocking apoptosis (20). The Bax protein controls cell death through activation of caspases 9 and 3 (21). Several natural compounds such as curcumin (22), resveratrol (23), (–)-epigallocatechin-3-gallate (EGCG) (24), capsaicin (25) and glycolic acid (26) have been reported to induce p53-dependent apoptosis in human ovarian, liver, prostate, leukemic and keratinocyte cancer cells contributing to the cancer cell growth inhibition. Previously, our preliminary study showed that berberine inhibited prostate cancer cell growth through a p53-dependent induction of apoptotic cell death (unpublished data).

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In the present study, we investigated whether berberine inhibits human neuroblastoma cell growth *via* an apoptotic cell death. We also investigated whether berberine-induced apoptosis is p53 dependent, causing a different growth inhibitory effect between human neuroblastoma SK-N-SH (p53-expressing) and SK-N-MC (p53-deficient) cells.

Materials and Methods

Cell culture and treatment. Human neuroblastoma cell lines SK-N-SH, p53-positive cells, and SK-N-MC, p53-negative cells, were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The neuroblastoma cell lines were cultured as monolayer in DMEM high glucose supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 mg/ml penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA), and maintained in an incubator with humidified atmosphere air 5% CO_2 at 37˚C. The cortical neuronal stem cells were prepared and cultured as previously described (27). In brief, embryonic neuronal stem cells were isolated from Sprague-Dawley pregnant rats sacrificed by cervical dislocation and whose embryos were removed on gestation days 14, 16 and 18. They were cultured in DMEM and Ham's F12 mixture media (1:1 mixture, Sigma, MO) with 10% NuSerum (NuSerum contains 25% newborn calf serum; Becton Dickinson, MA), 100 mg/ml penicillin-streptomycin and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO_2 at 37˚C. Berberine chloride was purchased from Sigma Aldrich (St. Louis, MO, USA). In all treatments, berberine was dissolved in a small amount of dimethyl sulfoxide (DMSO) and made up to a maximum final concentration of 0.05% (v/v) in the complete cell culture medium.

p53 siRNA transfection. Oligonucleotides of p53 siRNA were purchased from Samchully Pharm. Co. (Seoul, Korea). The sequence of the p53 oligonucleotide was: sense 5' (CUACUUCC UGAAAACAACG)d(TT)3' and anti-sense 5'(CGUUGUUUUCA GGAAG UAG)d(TT)3'. siRNA oligonucleotides were transfected using the siPORT™ NeoFX™ (Ambion, Cambridgeshire, United Kingdom). In preliminary studies, cells were treated with 10-200 nM of p53 siRNA according to the manufacturer's instruction in order to determine the optimum concentration to down-regulate the target genes. In later studies, cells were transfected with 10, 50 or 100 nM siRNA for 4 h, and then the cells were grown for a further 48 h in normal medium. The effect of siRNA treatment on sensitivity to apoptosis was assessed by flow cytometry 48 h after berberine treatment. The effect on cell viability of p53 siRNA treatment was determined by cell viability test 24 and 48 h after berberine treatment. Treatment of cells with the selective p53 inhibitor, pifithrin-α (Calbiochem, CA, USA), was performed by pretreatment of the cells with the inhibitor 24 h prior addition of berberine.

Cell viability assay. Cell viability assay was performed as described previously (27). Cells were plated in 96-well plates, and subconfluent cells were subsequently treated with berberine (0, 5, 10, 20, and 50 μM) for 24, 48, and 72 h. After treatment, cell viability was measured by Cell Counting Kit-8 (CCK-8) system (Dojindo, Kumamoto, Japan) using Dojindo's tetrazolium salt, WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction.

Cell cycle analysis by flow cytometry. Subconfluent cells were treated with berberine (0, 5, 10, 20, and 50 μM) in culture medium for 48 h and 50 μM concentration of berberine in complete medium for 0, 6, 12, 24, 48, and 72 h. Flow cytometric analysis was carried out as previously described (27). The cell cycle distribution was determined using a FACSCalibur instrument (BD Biosciences, San Jose, CA, USA).

Apoptosis evaluation. Apoptosis assays were performed in the berberine treated cells by using flow cytometric analysis and by fluorescence microscopy after the terminal nucleotidyl transferase-mediated nick-end labeling (TUNEL) with the *in situ* Cell Death Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The cells were then observed through a fluorescence microscope (magnification ×400, Leica Microsystems AG, Wetzlar, Germany). TUNEL staining assay was carried out as previously described (27).

Immunofluorescence staining. Immunofluorescence staining was performed as described previously (27). The cells were cultured with medium containing berberine (10 and 50 μM) or vehicle for 48 h. Primary polyclonal antibodies for p53 (1:400 dilution) and anti-rabbit-biotinylated secondary antibodies Alexa Fluor 568 (Molecular Probes Inc., Eugene, OR, USA) (1:200 dilution) were used. Upon nuclear stain and mounting in antifade medium with DAPI (Vector Laboratories, Inc.), immunofluorescence images were acquired using a confocal laser scanning microscope (TCS SP2; Leica Microsystems AG, Wetzlar, Germany) equipped with a 630 × oil immersion objective.

Western blot analysis. Western blot analysis was carried out as described previously (27). The cells were harvested and homogenized in lysis buffer Pro-Prep (iNtRON Biotechnology Co., GyeongGi-do, Korea). The membrane was incubated with specific antibody for p53 (1:500), CDK4 (1:1000), CDK2 (1:1000), cyclin D1 (1:1000), cyclin E (1:1000), p21 (1:500), Bcl-2 (1:1000), Bax (1:500), PARP (1:1000), and cleaved caspase-3 (1:1000) antibodies and horseradish peroxidase-conjugated IgG secondary antibody (Santa Cruz Biotech, Santa Cruz, CA). Immunoreactive proteins were detected with the ECL Western blotting detection system. The relative density of the protein bands was scanned by densitometry using MyImage (SLB, Seoul, Korea) and quantified by Labworks 4.0 software (UVP Inc., Upland, CA, USA).

Statistics. Data were analyzed using one-way analysis of variance (ANOVA) followed by post *hoc* Dunnet's test. A *p*-value of less than 0.05 was considered to be statistically significant.

Results

Effect of berberine on cell growth in neuroblastoma cells. Berberine (Figure 1A) was studied to assess its effect on the growth of human neuroblastoma SK-N-SH and SK-N-MC cells and cortical neuronal cells. The cells were treated with different concentrations of berberine (0, 5, 10, 20, 50 and 100 μM) for 24, 48 and 72 h (Figure 1B). Berberine inhibited cell proliferation of neuroblastoma cells at a concentration between 5 to 100 μM. However, the p53-expressing cell line SK-N-SH was more susceptible to berberine (IC_{50}=37 μM) than were

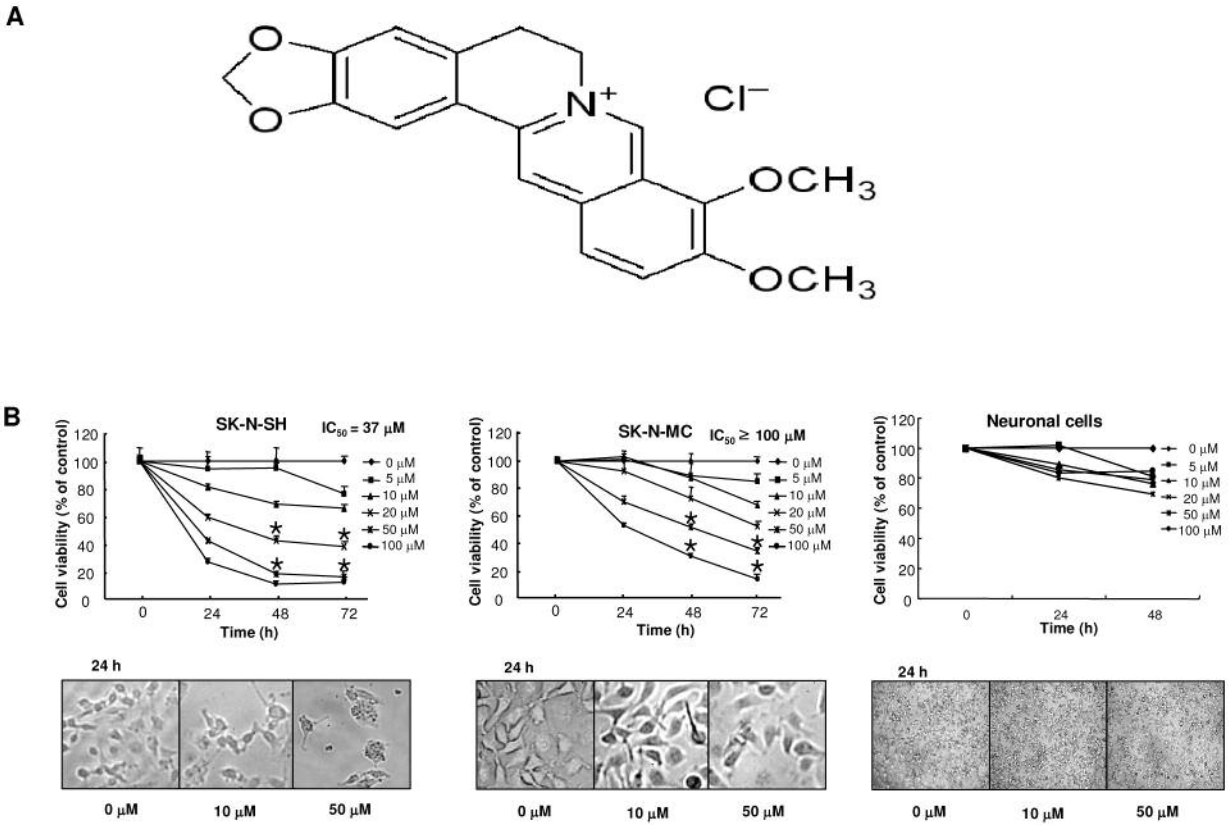

Figure 1. *Effect of berberine on cell viability in human neuroblastoma cells. A) Chemical structure of berberine. B) Concentration- and time-dependence of berberine effect on the cell viability. As detailed in Materials and Methods, the cells were treated with berberine (5-100 μM) for 24, 48, and 72 h. After 24 h of berberine treatment, the morphology cells was observed under phases contrast microscope (magnification, ×200). Results are the mean of three experiments in duplicate. *Significantly different from untreated control group (p<0.05).*

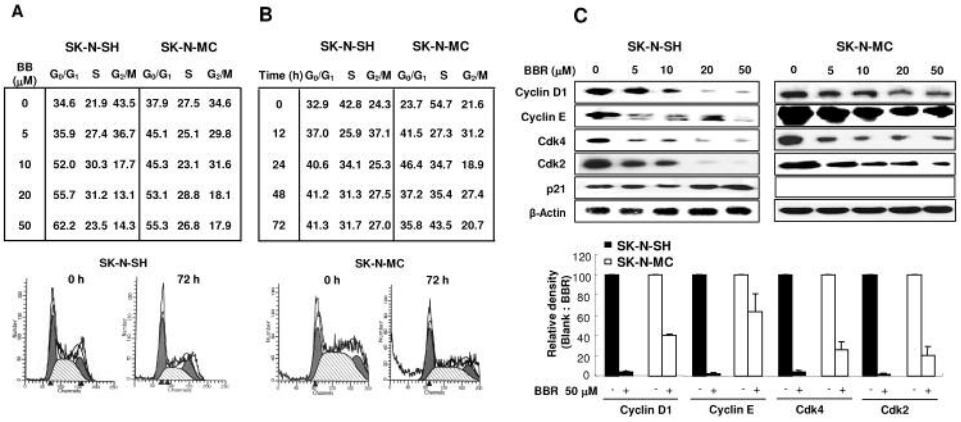

Figure 2. *Effect of berberine on cell cycle progression and expression of cell cycle regulatory proteins of human neuroblastoma cells. A) Concentration-dependent cell cycle progression. Cells were treated with 0, 5, 10, 20, and 50 μM of berberine in complete medium. After 48 h of treatment, cells were harvested and digested with RNase A. Cellular DNA was stained with propidium iodide (PI) and flow cytometric analysis was performed to analyze the cell cycle distribution, as detailed in the Materials and Methods. B) Time-dependent cell cycle progression. The cells were treated with 50 μM concentration of berberine in complete medium for 0, 6, 12, 24, 48, and 72 h. The cells were harvested and analyzed for DNA content by PI staining. Similar results were obtained from three independent experiments. C) Effect of berberine on the levels of expression of cell-cycle-related proteins (cyclins, Cdks, and p21) determined using Western blot analysis. The cells were treated with or without berberine for 24 h. Total cell lysates were processed for Western blot analysis as described in Materials and Methods. β-Actin was used as an internal control. The relative density was analyzed by densitometry, and the values were expressed as the mean ± SD of three experiments with duplicates.*

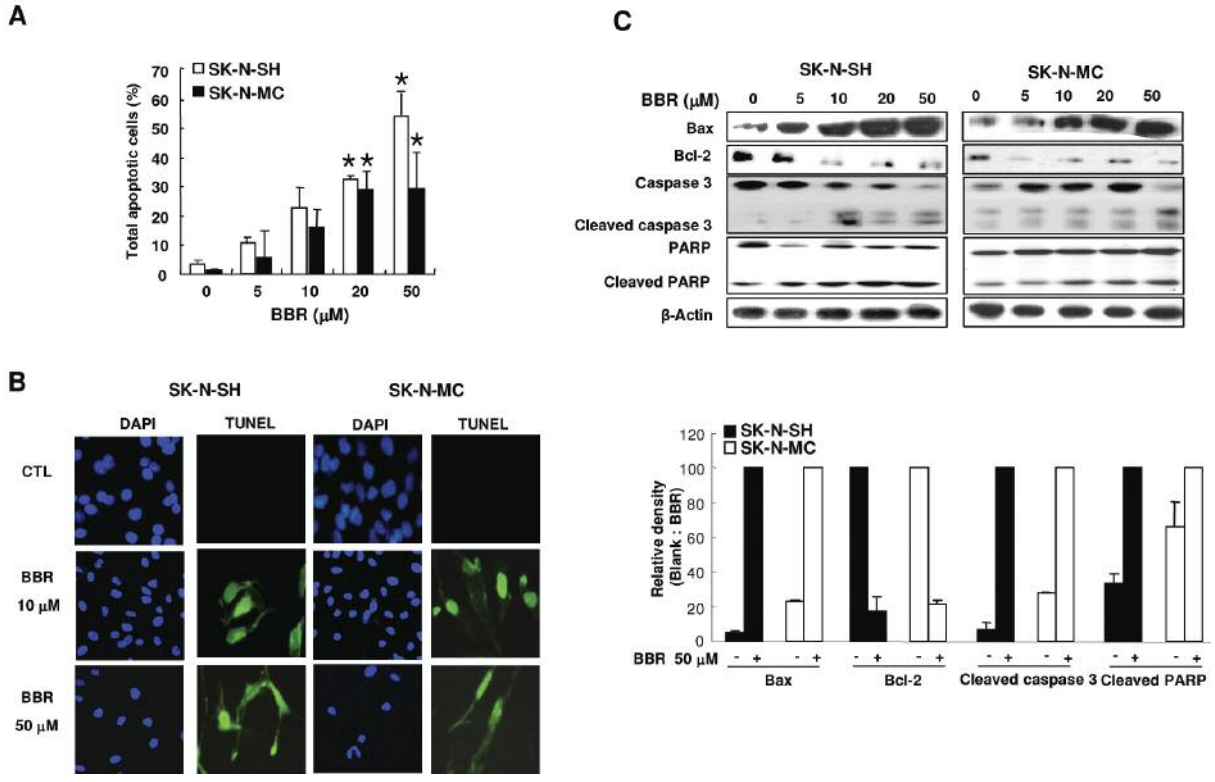

Figure 3. *Effect of berberine (BBR) on apoptosis and expression of apoptotic proteins in human neuroblastoma cells. A) Quantification of apoptosis by flow cytometric analysis. The cells were treated with berberine 0, 5, 10, 20, and 50 μM for 48 h, and then labeled with TUNEL solution. The data shown here are from typical experiments repeated three times. B) TUNEL micrographs. The green color marks TUNEL-labeled cells. Total number of apoptotic cells in a given area was determined with DAPI in TUNEL-labeled cells (magnification, ×400). C) Effect of berberine on the levels of expression of apoptosis regulatory molecules (Bax, Bcl-2, caspase 3, and PARP) was determined using Western blot analysis. The cells were treated with different concentration of berberine for 24 h. As detailed in Materials and Methods, the total cell lysates were prepared for Western blot analysis. The relative density was analyzed by densitometry, and the data were expressed as the mean ± SD of three experiments with duplicates. *Significantly different from untreated control group (p<0.05).*

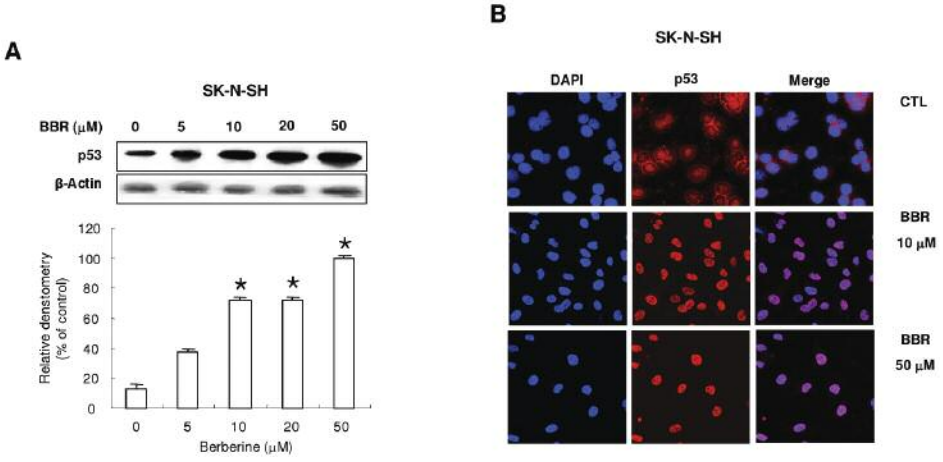

Figure 4. *Effect of berberine (BBR) on p53 expression and translocation into nucleus in human neuroblastoma cells. A) Effect of berberine on the level of p53 protein in the nuclear fraction. The cells were treated with berberine (0, 5, 10, 20, and 50 μM) for 24 h and then harvested and lysed as detailed in Materials and Methods. Values of the relative densities were expressed as the mean ± SD of three experiments with duplicates. B) The nuclear translocation of p53 was determined by immunofluorescence confocal laser scanning microscopy. After treatment with berberine for 24 h, p53 was translocated into the nucleus. The cell fluorescence was developed with Alexa-Fluor 568 (1:100) for 1 h. Similar results were obtained in three independent experiments (magnification, ×630). *Significantly different from untreated control group (p<0.05).*

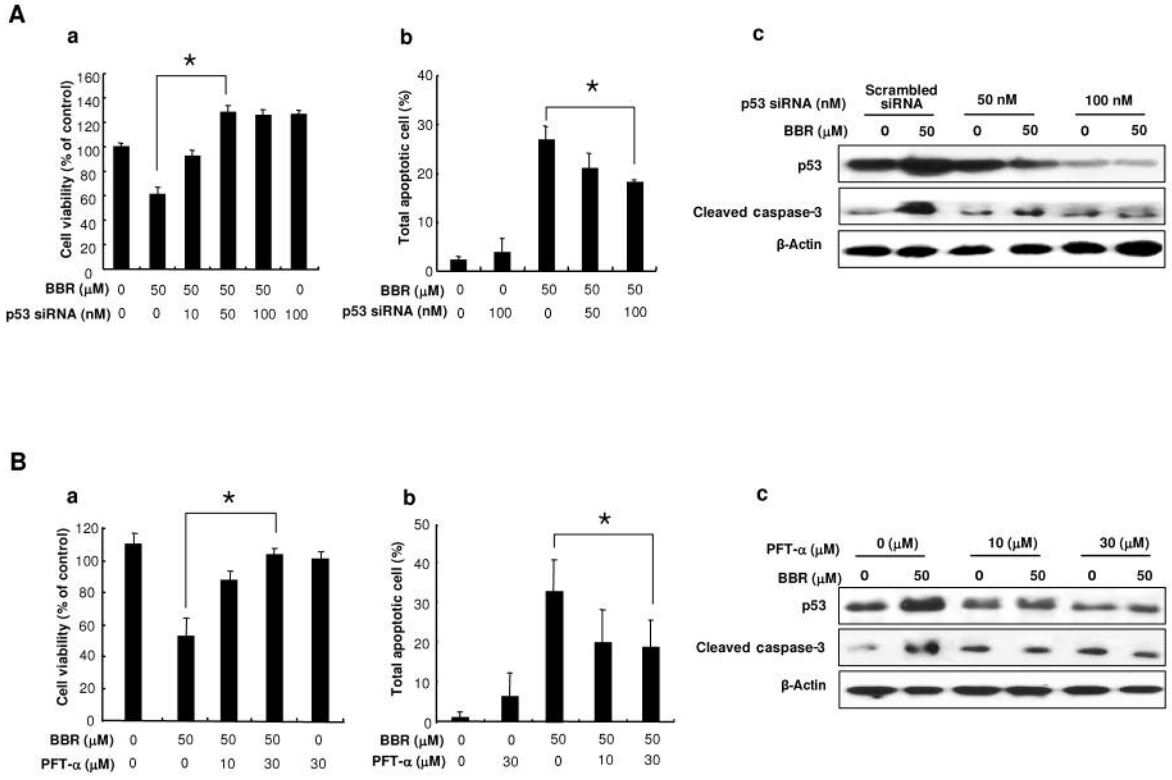

Figure 5. *Effect of p53 inhibitor or p53 siRNA on the cell viability, apoptotic cell death and caspase-3 expression in berberine-treated SK-N-SH cells. A) Cell viability (a) and apoptosis (b) of cells transfected with p53 siRNA. Cells were transfected with p53 siRNA for 4 h, and grown for 48 h, then treated with berberine for 24 h. B) Cell viability (a) and apoptosis (b) in p53-expressing cells treated with the p53 inhibitor (PFT-α). Cells were pretreated with the inhibitor for 24 h prior to the addition of berberine. Data are expressed as a percentage of control cells (scrambled siRNA-transfected cells or untreated) as the mean ± SD of three replicates. Cell viability was determined by the CCK-8 kit. Quantification of apoptosis was determined by DAPI and TUNEL double staining assay. The data are expressed as percentage of control cells as the mean ± SD of three experiments with duplicates. Apoptotic protein expression was determined by Western blot in cells transfected with p53 siRNA (Ac) or treated with PFT-α (Bc). Using the same protocol as for (a) and (b), Western blot analysis was used to detect the changes in p53 and cleaved caspase 3 protein expression levels. Results are representative of three independent experiments. *Significantly different from berberine single treated control ($p<0.05$).*

SK-N-MC, p53-negative cells (IC_{50}≥100 μM). Contrasting with the effect of berberine on cancer cells, normal cortical neuronal cells were not affected with the same concentrations of berberine.

Berberine induced G_0/G_1-phase cell cycle arrest and decreased the levels of G_0/G_1 regulatory proteins. We determined the possible inhibitory effect of berberine on the cell cycle progression. As summarized in Figure 2, berberine arrested the growth of a significantly higher number of cells in the G_0/G_1 phase in a concentration-, but not time-dependent manner in the treated cells (Figure 2A and B). The number of cells in G_2/M- and S-phase also decreased in the two cell lines. After 6 h of treatment, the G_0/G_1-phase of cells gradually increased up to 72 h. Interestingly, treatment with berberine (50 μM) for 48 h resulted in a significantly higher number of cells in the G_0/G_1-phase in SK-N-SH (62%) cells compared with SK-N-MC (55%) cells. These results suggest that inhibition of cell proliferation in both neuroblastoma cell types may be associated with the induction of G_0/G_1 arrest through a process more effective in p53-expressing cells.

The effect of berberine on cell cycle-regulatory molecules operative in the G_0/G_1-phase was then determined. Analysis of the cyclin/Cdk complexes shows a significant reduction in cyclin D1, cyclin E, Cdk2, and Cdk4 expression in the two cell lines. This inhibition of the expression of G_0/G_1 regulatory protein was much greater in p53-expressing cells than in the p53-negative cells (Figure 2C). CDKIs are well known to interfere with cell cycle progression inducing a phase-specific cycle arrest. Thus, the protein level of certain CDKI family member, such as p21, a crucial protein in the regulation of G_0/G_1-phase progression, was determined by Western blot analysis. Berberine treatment up-regulated the expression levels of p21 in SK-N-SH cells, however, p21 protein could not be detected in SK-N-MC cells. These

results imply that the down-regulation of cyclin D1, cyclin E, Cdk2, and Cdk4 protein expressions may be responsible for the G0/G1 cell cycle arrest induced by berberine. Regulation of p21 proteins may also be involved in berberine-triggered p53-dependent arrest of the SK-N-SH cells in the G_0/G_1 phase of the cell cycle.

Berberine induced apoptosis and apoptotic protein expression. To assess whether cell cycle arrest resulted in the induction of apoptosis, apoptosis was quantified in neuroblastoma cells. The cells were treated with different concentrations of berberine (0, 5, 10, 20, and 50 μM) for 48 h. Treatment of SK-N-SH and SK-N-MC cells resulted in an increase of apoptosis in a concentration-dependent manner (Figure 3A). The increase in the apoptotic cells percentage was quantified using confocal microscopy. Berberine increased apoptosis in both neuroblastoma cells (Figure 3B). However, consistent with results of the cell viability test and cell cycle arrest, berberine induced a greater apoptosis in p53-expressing SK-N-SH cells (54.3% by 50 μM) than in p53-deficient SK-N-MC cells (29.4% by 50 μM) (Figure 3A).

Since Bax and Bcl-2 play a crucial role in apoptosis under p53 control, we determined the effect of berberine on the level of Bax and Bcl-2 expression in the two cell lines. The Western blot analysis showed a significant increase of the pro-apoptotic Bax protein (Figure 3C). In contrast, the anti-apoptotic Bcl-2 protein significantly decreased. Alteration in Bax/Bcl-2 is known to initiate caspase signaling, a p53 target signal. The involvement of caspase-3 during berberine-mediated apoptotic death was investigated. As shown by the immunoblot analysis (Figure 3C), berberine treatment resulted in a significant increase of the expression of cleaved caspase 3 and cleaved PARP, an indication of the activation of caspase-3. However, the extent of expression changes of Bax/Bcl-2 and cleaved caspase-3 was much significant in SK-N-SH cells than in SK-N-MC.

Berberine induced p53 translocation to nucleus in SK-N-SH cells. p53 is a cellular gatekeeper for the cell growth and division (28). It has been shown that p53 can regulate cell cycle arrest and apoptosis in a variety of cells. Since berberine induced more changes in the p53-expressing SK-N-SH cells, its effect on p53 translocation into nucleus was investigated. Immunoblot analysis shown that nuclear translocation of p53 proteins was highly increased in a concentration-dependent manner (Figure 4A). Confocal microscopy of immunofluorescence-stained p53 shows that p53 expression was significantly increased by berberine (Figure 4B). The increased p53 proteins were concentrated on the nuclei.

Knock-down of p53 reduced berberine-induced cell growth inhibition and expression of apoptosis-related proteins in SK-N-SH cells. To investigate whether berberine-induced apoptosis is p53 dependent or not, two sets of experiments were carried out. First, p53 protein expression in the SK-N-SH cells was knocked down by p53 siRNA treatment. Second, cells were treated with berberine in the presence of PFT-α, a compound which inhibits the accumulation and/or the transcriptional activity of p53 (29). As shown in Figure 5Aa, transfection of p53 siRNA significantly suppressed berberine-induced cell growth inhibition. Pretreatment with PFT-α also suppressed the berberine-induced cell growth inhibition (Figure 5Ba). The increase of the number of apoptotic cells was also reduced when cells were treated with berberine and p53 siRNA or PFT-α (Figure 5Ab, 5Bb). In agreement with these findings, the expression of p53 and cleaved caspase 3 protein in cells treated with berberine and p53 siRNA or PFT-α reversed the berberine effect (Figure 5Ac, 5Bc).

Discussion

In this study, we demonstrated that berberine (5-50 μM) inhibited cell growth in neuroblastoma SK-N-SH and SK-N-MC cells through induction of apoptosis. Moreover, we also demonstrated that the p53-expressing SK-N-SH cell line, was more sensitive to berberine. This suggests that activation of p53-dependent apoptosis is implicated in berberine-induced p53-mediated neuroblastoma cell growth inhibition.

The most common anti-apoptotic lesion in cancer is the inactivation of p53 tumor-suppressor gene (16). It has been well established that p53 plays multiple tumor suppressive roles in stressed cells to regulate cell cycle G1 and G2 arrest or apoptosis (30). In most cases, induction of *p53* leads to an irreversible inhibition of cancer cell growth, mainly by activating apoptotic pathways. Several naturally occurring anti-tumor agents are able to induce a cell cycle arrest followed by apoptosis of the cancer cells in a p53-dependent manner. [See for examples: human gastric cancer cell death by arsenic trioxide (31), bladder cancer T24 cell with ellagic acid (32), lung cancer A549 cell with plumbagin (33), leukemia cell with capsaicin (25), gastric carcinoma SNU-5 cells with berberine (12).] We also demonstrated that berberine-induced apoptosis of neuroblastoma cells is p53-dependent by comparison of the vulnerability of p53-positive SK-N-SH cells with p53-negative SK-N-MC cells. Berberine inhibited cell growth of SK-N-SH and SK-N-MC. However, the growth inhibitory effect of berberine was more effective against the p53-expressing cell line. We also found that berberine increased the translocation of p53 proteins into the nucleus in SK-N-SH cells. In addition, the inhibition of p53 with a p53 siRNA or PFT-α, a specific p53 inhibitor, attenuated the berberine-dependent apoptosis in SK-N-SH cells. We recently found a similar effect of berberine towards prostate cancer cells (unpublished data).

Molecular analyses of human cancer have revealed that cell cycle regulators are frequently involved in most of the common malignancies. Among them, $p21^{Cip1/Waf1}$ is an inhibitor of cyclin-dependent kinases 2, 4 and 6. Its up-regulation causes cells to undergo a G_1 arrest. Our data showed that, consistent with the greater effect on cell growth inhibition, the G_0/G_1-phase arrest of SK-N-SH cells was greater than that of SK-N-MC cells which lack p21 (Figure 2C). In addition, a greater change of cyclin D1, cyclin E, Cdk2 and Cdk4 in p53-expressing cells was observed, being correlated with the induction of apoptosis. Furthermore, p53 siRNA and inhibitor of p53 suppressed the berberine-induced apoptosis of SK-N-SH cells. This was accompanied by activation of caspase-3, a p53-mediated apoptosis regulatory protein. In agreement with the caspase-3 activation in the p53-dependent berberine-induced apoptosis of neuroblastoma, involvement of the activation of caspases has been demonstrated in berberine-treated human epidermoid carcinoma A431 cells, prostate cancer cells, promonocytic U937 cancer cells, leukemia HL-60 cells, and human hepatoma cells (9-11, 13, 34).

Other mechanism such as generation of reactive oxygen species (ROS) could be involved in the berberine-induced apoptotic cell death in neuroblastoma cells. In fact, berberine induced SW620 colon cancer cell and also prostate cancer cell (PC-3) death *via* generation of ROS (35, 36).

Very few drugs have been developed for the treatment of malignant brain tumors. Berberine is known to pass through the blood-brain barrier (37, 38). We have also found that berberine penetrated into the nucleus of both SK-N-SH and SK-N-MC cells (data not shown). It could be therefore effective for the treatment of neuroblastoma *in vitro*.

Acknowledgements

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References

1 Takase H, Yamamoto K, Ito K and Yumioka E: Pharmacological studies on antidiarrheal effects of berberine and geranin herb. Nippon Yakurigaku Zasshi *2*: 101-112, 1993.

2 Huang WM, Wu ZD and Gan YQ: Effects of berberine on ischemic ventricular arrhythmia. Zhonghua Xin Xue Guan Bing Za Zhi *5*: 300-301, 1989.

3 Kuo CL, Chi CW and Liu TY: The anti-inflammatory potential of berberine *in vitro* and *in vivo*. Cancer Lett *203*: 127-137, 2004.

4 Hano K: Pharmacological studies on metabolism of cancer tissues: pharmacological studies on carcinostatic effects of some plant components and their derivatives. Gann *48*: 443-450, 1957.

5 Hoshi A, Ikekawa T, Ikeda Y, Shirakawa S and Iigo M: Antitumor activity of berberrubine derivatives. Gann *67*: 321-325, 1976.

6 Liu LF: DNA topoisomerase poisons as antitumor drugs. Ann Rev Biochem *58*: 351-375, 1989.

7 Mazzini S, Bellucci MC and Mondelli R: Mode of binding of the cytotoxic alkaloid berberine with the double helix oligonucleotide d(AAGAATTCTT)(2). Bioorg Med Chem *11*: 505-514, 2003.

8 Qin Y, Pang JY, Chen WH, Cai Z and Jiang ZH: Synthesis, DNA-binding affinities, and binding mode of berberine dimers. Bioorg Med Chem *14*: 25-32, 2006.

9 Mantena SK, Sharma SD and Katiyar SK: Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. Mol Cancer Ther *5*: 296-308, 2006

10 Mantena SK, Sharma SD and Katiyar SK: Berberine inhibits growth, induces G1 arrest and apoptosis in human epidermoid carcinoma A431 cells by regulating Cdki-Cdk-cyclin cascade, disruption of mitochondrial membrane potential and cleavage of caspase 3 and PARP. Carcinogenesis *27*: 2018-2027, 2006

11 Jantova S, Cipak L and Letasiova S: Berberine induces apoptosis through a mitochondrial/caspase pathway in human promonocytic U937 cells. Toxicol In Vitro *21*: 25-31, 2007.

12 Lin JP, Yang JS, Lee JH, Hsieh WT and Chung JG: Berberine induces cell cycle arrest and apoptosis in human gastric carcinoma SNU-5 cell line. World J Gastroenterol *12*: 21-28, 2006.

13 Hwang JM, Kuo HC, Tseng TH, Liu JY and Chu Cy: Berberine induces apoptosis through a mitochondria/caspases pathway in human hepatoma cells. Arch Toxicol *80*: 62-73, 2006.

14 Fukuda K, Hibiya Y, Mutoh M, Koshiji M, Akao S and Fujiwara H: Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells. J Ethnopharmacol *2*: 227-233, 1999.

15 Lin JG, Chung JG, Wu LT, Chen GW, Chang HL and Wang TF: Effects of berberine on arylamine *N*-acetyltransferase activity in human colon tumor cells. Am J Chin Med *27*: 265-275, 1999.

16 Hainaut P and Hollstein M: p53 and human cancer: the first ten thousand mutations. Adv Cancer Res *77*: 81-137, 2000.

17 Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R and Beach D: P21 is a universal inhibitor of cyclin kinases. Nature *366*: 701-704, 1993.

18 Vousden KH and Lu X: Live or let die: the cell's response to p53. Nat Rev Cancer *2*: 594-604, 2002.

19 Hoffman WH, Biade S, Zilfou JT, Chen J and Murphy M: Transcriptional repression of the anti-apoptotic *survivin* gene by wild-type p53. J Biol Chem *277*: 3247-3257, 2002.

20 Olitvai ZN, Milliman CL and Korsmeyer SJ: Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell-death. Cell *74*: 609-619, 1993.

21 Wang X: The expanding role of mitochondria in apoptosis. Genes Dev *15*: 2922-2933, 2001.

22 Weir NM, Selvendiran K, Kutala VK, Tong L, Vishwanath S, Rajaram M, Tridandapani S, Anant S and Kuppusamv P: Curcumin induces G(2)/m arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by modulating Akt and p38 MAPK. Cancer Biol Ther *6*: 178-184, 2007.

23 Kuo PL, Chiang LC and Lin CC: Resveratrol-induced apoptosis is mediated by p53-dependent pathway in Hep G2 cells. Life Sci *72*: 23-34, 2002.

24 Hastak K, Gupta S, Ahmad N, Agarwal ML and Mukhtar H: Role of p53 and NF-kappaB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. Oncogene *22*: 4851-4859, 2003.

25 Ito K, Nakazato T, Yamamoto K, Miyakawa Y, Yamada T, Hozumi N, Segawa K, Ikeda Y and Kizaki M: Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: Implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer Res *64*: 1071-1078, 2004.

26 Ahn KS, Park KS, Jung KM, Jung HK, Lee SH, Chung SY, Yang KH, Yun YP, Pyo HB, Park YK, Yun YW, Kim DJ, Park SM and Hong JT: Inhibitory effect of glycolic acid on ultraviolet B-induced c-fos expression, AP-1 activation and p53-p21 response in a human keratinocyte cell line. Cancer Lett *186*: 125-135, 2002.

27 Son DJ, Park MH, Chae SJ, Moon SO, Lee JW, Song HS, Moon DC, Kang SS, Kwon YE and Hong JT: Inhibitory effect of snake venom toxin from *Vipera lebetina turanica* on hormone-refractory human prostate cancer cell growth: induction of apoptosis through inactivation of nuclear factor kappaB. Mol Cancer Ther *6*: 675-683, 2007.

28 Levine AJ: p53, the cellular gatekeeper for growth and division. Cell *88*: 323-331, 1997.

29 Komarov PG, Komarova EA, Kondratov RV, Christov-Tselkov K, Chemov MV and Gudkov AV: A chemical inhibitor of p53 that protects mice from the side-effects of cancer therapy. Science *285*: 1733-1737, 1999.

30 Kastan MB, Canman CE and Leonard CJ: P53, cell cycle control and apoptosis: implications for cancer. Cancer Metastasis Rev *14*: 3-15, 1995.

31 Jiang XH, Wong BC, Yuen St, Jiang SH, Cho CH, Lai KC, Lin MC, Kung HF and Lam SK: Arsenic trioxide induces apoptosis in human gastric cancer cells through up-regulation of p53 and activation of caspase-3. Int J Cancer *91*: 173-179, 2001.

32 Li TM, Chen GW, Su CC, Lin JG, Yeh CC, Cheng KC and Chung JG: Ellagic acid induced p53/p21 expression, G1 arrest and apoptosis in human bladder cancer T24 cells. Anticancer Res *25*: 971-979, 2005.

33 Hsu YL, Cho CY, Kuo PL, Huang YT and Lin CC: Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) induces apoptosis and cell cycle arrest in A549 cells through p53 accumulation *via* c-Jun NH2-terminal kinase-mediated phosphorylation at serine 15 *in vitro* and *in vivo*. J Pharmacol Exp Ther *318*: 484-494, 2006.

34 Lin CC, Kao ST, Chen GW, Ho HC and Chung JG: Apoptosis of human leukemia HL-60 cells and murine leukemia WEHI-3 cells induced by berberine through the activation of caspase-3. Anticancer Res *26*: 227-242, 2006.

35 Hsu WH, Hsieh YS, Kuo HC, Teng CY, Huang HI, Wang CJ, Yang SF, Liou HS and Kuo WH: Berberine induced apoptosis in SW620 human colonic carcinoma cells through generation of reactive oxygen species and activation JNK p38 MAPK and FASL. Arch Toxicol *81*: 719-728, 2007.

36 Meeran SM, Katiyar S and Katiyar SK: Berberine-induced apoptosis in human cancer cells is initiated by reactive oxygen species generation. Toxicol Appl Pharmacol *229*: 33-43, 2008.

37 Ozaki Y, Suzuki H and Satale M: Comparative studies on concentration of berberine in plasma after oral administration of Coptidis rhizoma extract, its cultured cells extract, and combined use of these extracts and Glycyrrhizae radix extract in rats. Yakugaku Zasshi *113*: 63-69, 1993.

38 Wang X, Wang R, Xing D, Su H, Ma C, Ding Y and Du L: Kinetic difference of berberine between hippocampus and plasma in rat after intravenous administration of Coptidis rhizoma extract. Life Science *77*: 3058-3067, 2005.

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