Berberine Induces Apoptosis in Human HSC-3 Oral Cancer Cells *via* Simultaneous Activation of the Death Receptor-mediated and Mitochondrial Pathway

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Abstract. *Evidence has accumulated that berberine is able to induce cell cycle arrest and apoptosis in many human cancer cell lines. However, there is no available information on the effects of berberine on human oral squamous cell carcinoma. In this study, the effects of berberine on cell growth, apoptosis and cell cycle regulation in human oral squamous carcinoma HSC-3 cells were examined. Berberine induced dose- and time-dependent irreversible inhibition of cell growth and cellular DNA synthesis. This was also confirmed by phase-contrast microscopy which showed that berberine induced morphological changes in HSC-3 cells. Propidium iodide/annexin V staining for flow cytometric analysis showed that berberine-induced apoptosis correlated with caspase-3 activation. Flow cytometric studies of the cell cycle distribution showed that berberine induced mainly G0/G1-phase arrest. Flow cytometric examinations also showed that berberine induced reactive oxygen species (ROS) and Ca^{2+} production, as well as the dysfunction of mitochondrial membrane potential (MMP), which were correlated with apoptosis. In conclusion, our data support that berberine initially induces an endoplasmic reticulum stress response based on ROS and Ca^{2+} production which is followed by dysfunctions of the mitochondria, resulting in apoptosis of these oral cancer HSC-3 cells. Prolonged exposure of the HSC-3 cells to berberine causes increased apoptosis through reduced levels of MMP, release of cytochrome c and activation of caspase-3.*

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Oral and oropharyngeal cancers are a major health problem worldwide, accounting for over 300,000 cases annually (1). Incidence rates of oral cancer have been rising in most regions of the world (2), including Taiwan, and it is more common among males than females (1). Tobacco and alcohol consumption are recognized as being major factors for the development of oral cancers (3-5). However, other factors, such as low carotenoid and vitamin A diets, poor oral hygiene and indoor air pollution, are also involved in oral cancer (6-9). In Taiwan, there is another factor, betel quid chewing, which is one of the important factors of oral cancer. The therapies for oral cancer include irradiation, surgery, or chemotherapy. However, these methods are still unsatisfactory because of their side-effects.

Berberine [$(C_{20}H_{18}NO_4)^+$], one of the components of the Chinese herb *Rhizoma coptidis*, is a type of isoquinoline alkaloid. *Rhizoma coptidis* has been used in Chinese medicine for a long time (10). Berberine has a wide range of pharmacological actions, such as antidiarrheal, antimicrobial, anticancer, antiinflammatory, and antiarrhythmic activities (11). Berberine was reported to improve insulin resistance (12) and lower hyperglycemia (13). In our previous studies, we had reported that berberine induced apoptosis in human gastric cancer (14) and leukemia cells (15). It was also reported that berberine inhibits growth, induces G1 arrest and apoptosis in human epidermoid carcinoma A431 cells *via* regulating the Cdki-Cdk-cyclin cascade, the disruption of the mitochondrial membrane potential and cleavage of

caspase-3 and poly ADP-ribose polymerase (PARP) (16). It has recently been reported that berberine induces apoptosis in human promonocytic U937 cells through a mitochondrial/caspase pathway (17). However, there is no available information to address berberine affecting human oral cancer cells, and the underlying mechanisms remain uncertain. Therefore, in the present studies, we examine the effect of berberine on human oral cancer HSC-3 cells.

Materials and Methods

Chemicals and reagents. Berberine, dimethyl sulfoxide (DMSO), potassium phosphates, propidium iodide (PI), ribonuclease-A, Tris-HCl, trypan blue and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). TE buffer was purchased from Merck Co. (Darmstadt, Germany). 2,7-Dichlorodihydro-fluorescein diacetate, $DiOC_6$ and Indo 1/AM were obtained from Calbiochem (Darmstadt, Germany). z-VAD-fmk was obtained from R&D Systems, Inc. (MN, USA). Dulbecco's modified Eagle's medium (DMEM), glutamine, fetal bovine serum (FBS) and penicillin-streptomycin, trypsin-EDTA were obtained from Gibco BRL (Grand Island, NY, USA).

Human oral squamous cell carcinoma cells (HSC-3). The HSC-3 cell line (human oral squamous cell carcinoma) was obtained from the Dr. Weng J.R. Laboratory, Kaoshung Medical University (Kaoshing, Taiwan, ROC). Cells were cultured at 37˚C under a humidified 5% CO_2 and 95% air atmosphere in DMEM containing 10% fetal calf serum (FCS) in 75 cm^3 tissue culture flasks 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin) and 1% glutamine, as described elsewhere (18).

Measurement of cell viability by using trypan blue exclusion and flow cytometry. HSC-3 cells were plated in 12-well plates at a density of $5x10^5$ cells/well and grown for 24 h. Berberine was added to cells to achieve a final concentration of 0, 5, 10, 25, 50 and 75 μM, while for the control regimen only DMSO (solvent) was added and the cells were grown for different periods of time at 37˚C, 5% CO_2 and 95% air. The trypan blue exclusion and flow cytometry protocols for determining cell viability were used as previously described (18, 19).

Flow cytometric analysis of apoptosis in HSC-3 cells after treatment with different concentrations of berberine. Approximately, $5x10^5$ HSC-3 cells/well in each well of a 12-well plate were incubated with berberine (0, 5, 10, 25, 50 and 75 μM) for different time periods before being harvested by centrifugation. The cells were fixed gently (drop by drop) in 70% ethanol (in PBS) in ice overnight at –20˚C and were then resuspended in PBS containing 40 μg/mL PI, 0.1 mg/mL RNase (Sigma) and 0/1% Triton X-100. After being kept in the dark for 30 min at 37˚C, the cells were transferred to a 5 ml tube, and were then analyzed with a flow cytometer (Becton-Dickinson, San Jose, CA, USA) equipped with an argon laser at 488 nm. Apoptosis was then determined and analyzed (18, 19).

Detection of reactive oxygen species (ROS) in HSC-3 cells by flow cytometry. HSC-3 cells were treated with berberine (0, 5, 10, 25, 50 and 75 μM) for 12 h to determine its effect on ROS production. The cells were harvested, washed twice, re-suspended in 500 μl of 2,7-dichlorodihydrofluorescein diacetate (DCDHF-DA) (10 μM) and incubated at 37˚C for 30 min and analyzed by flow cytometry, as described elsewhere (18, 19).

Determination of Ca^{2+} concentrations in HSC-3 cells by flow cytometry. HSC-3 cells were treated with berberine (0, 5, 10, 25, 50 and 75 μM) for 12 h to determine the changes of Ca^{2+} concentration. The cells were harvested and washed twice: once for apoptosis analysis and the other for re-suspension in Indo 1/AM (3 μg/ml), incubated at 37˚C for 30 min and then analyzed by flow cytometry, as described elsewhere (18, 19).

Determination of mitochondrial membrane potential in HSC-3 cells by flow cytometry. HSC-3 cells were treated with berberine (0, 5, 10, 25, 50 and 75 μM) for 12 h to determine the changes of mitochondrial membrane potential. The cells were harvested and washed twice, re-suspended in 500 μl of $DiOC_6$ (4 mol/L) and then incubated at 37˚C for 30 min and analyzed by flow cytometry (18, 19).

Detection of caspase-3 activity and apoptosis in HSC-3 cells by flow cytometry. The caspase-3 activity and apoptosis of the HSC-3 cells were determined by flow cytometry (Becton Dickinson, FACSCalibur) using PhiPhiLux-G_2D_2 ($4x10^{-4}$ mol/L) (OncoImmunin, Inc., MD, USA). Cells were pretreated with or without caspase-3 inhibitor (z-VAN-fmk) and then treated with or without 50 μM berberine for 24 h then to detect changes in caspase-3 activity and apoptosis The cells were harvested and washed twice, re-suspended in 50 μl PhiPhiLux-G2D2 ($4x10^{-4}$ mol/L), incubated at 37˚C for 30 min and then analyzed by flow cytometry, as described elsewhere (18, 19).

Western blotting for examining the effect of berberine on CDK1, Wee1, Cdc25, p53, Bcl-2 family proteins and cytochrome c of HSC-3 cells. The total proteins were collected from HSC-3 cells treated with or without various concentrations of berberine for 48 h before CDK1, wee1, CDC25, p53, the Bcl-2 family of proteins (Bcl-2, Bcl-xL, Bax, BAD and Bak) and cytochrome *c* were measured by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot, as described elsewhere (18, 19).

Statistical analysis. Student's *t*-test was used to analyze statistical differences between the berberine-treated and control groups. Significant differences between both groups were determined at $p<0.05$ and $p<0.01$.

Results

Effects of berberine on the cell viability of HSC-3 cells. The cell viability was significantly lower in the berberine-treated than in control groups. The effects of berberine on HSC-3 cells were dose-dependent (Figure 1A and B). Increasing the time of incubation led to a reduction of cell viability (Figure 1C). Apparently the effects of berberine on HSC-3 cells are also time-dependent.

Induction of apoptosis by berberine in HSC-3 cells. Apoptosis was detected by a PI staining and annexin V method after 48 h of continuous exposure to berberine (Figue 2A and B). As shown in Figure 2, berberine induced apoptosis in a concentration- and time-dependent manner.

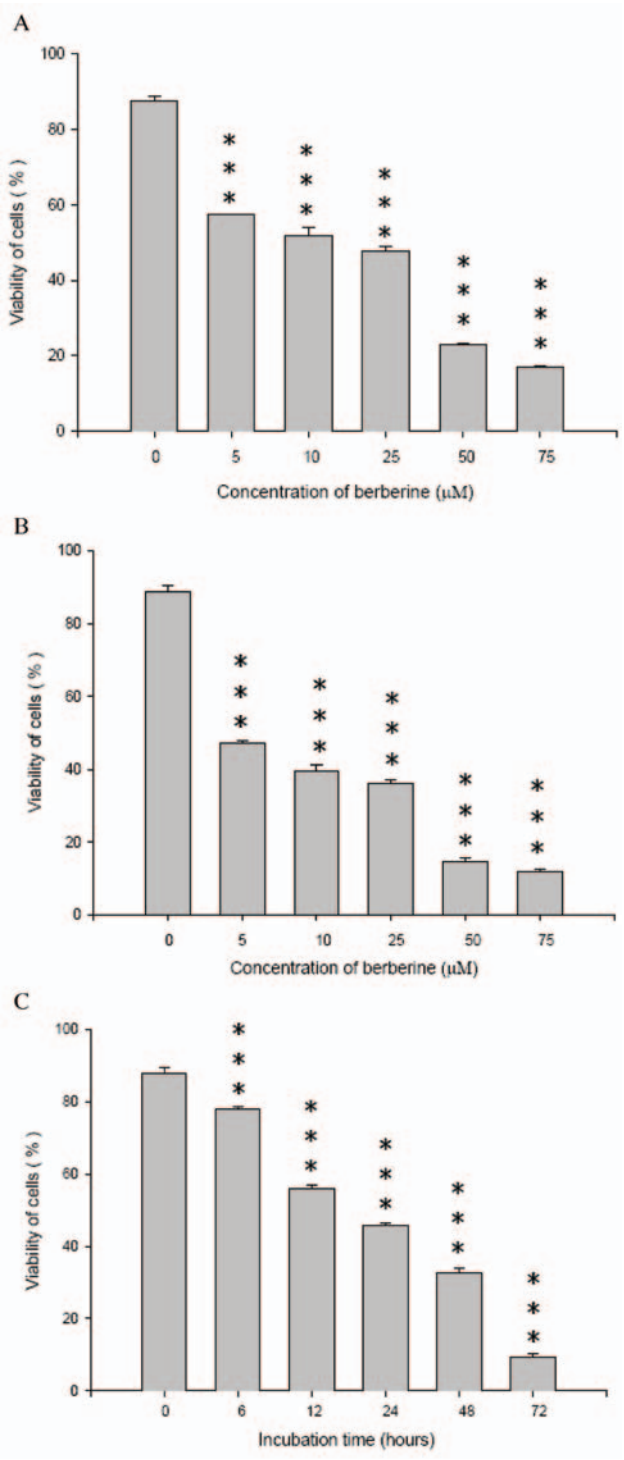

Figure 1. *The percentage of viable HSC-3 cells after berberine treatment. The HSC-3 cells (5x10^5 cells/well; 12-well plates) were plated in DMEM with different concentrations of berberine for 24 hours (panel A) and 48 hours (panel B) or 50 μM berberine for 0, 6, 12, 24, 48 and 72 hours (panel C). Cells were collected by centrifugation and the viable cells were determined by trypan blue exclusion and flow cytometry, as described in Materials and Methods. Each point is mean±S.D. of three experiments. Significantly different from the control was at *p<0.05 and ***p<0.01.*

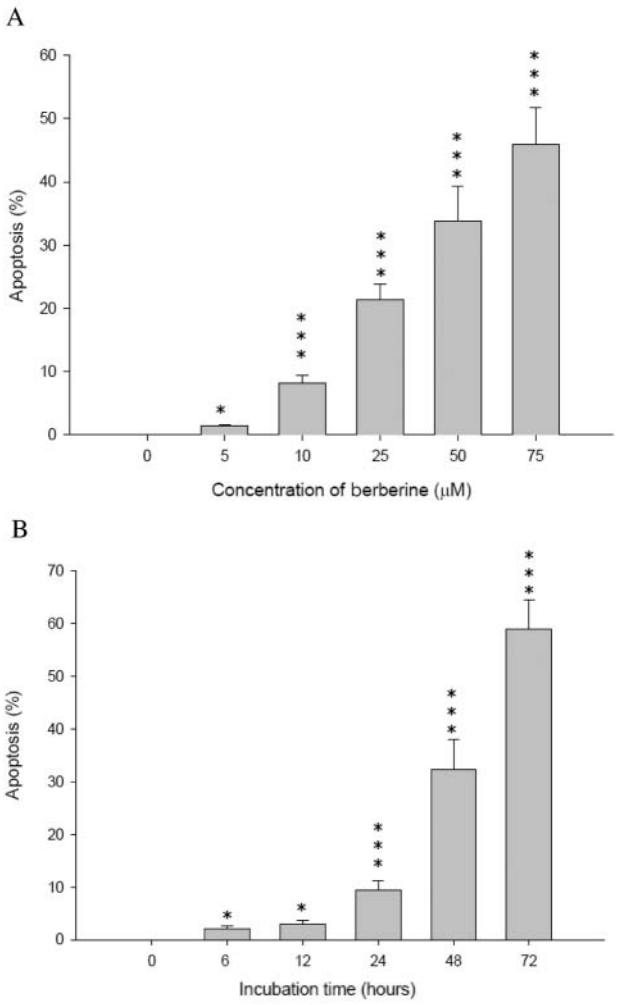

Figure 2. *Flow cytometric analysis of the effects of berberine on apoptosis in HSC-3 cells. The HSC-3 cells were exposed to various concentrations of berberine for 48 h (panel A: the percent of cells in apoptosis) or 50 μM berberine for 0, 6, 12, 24, 48 and 72 h (panel B: the percent of cells in apoptosis) and the cells were harvested and analyzed for apoptosis by flow cytometry as described in Materials and Methods. Data represents mean±S.D. of three experiments. Significantly different from the control was at *p<0.05 and ***p<0.01.*

Effects of berberine on the production of reactive oxygen species (ROS) on HSC-3 cells. The percentage of ROS was significantly higher in the berberine-treated than in the control groups. The effects of berberine on the taking up of the DCFH-DA dye by HSC-3 cells were dose-dependent (Table I).

Effects of berberine on the production of Ca^{2+} on HSC-3 cells. The Ca^{2+} concentration was significantly higher in the berberine-treated than in control groups as shown by Indo-1/AM staining. The effects of berberine on the taking up of the Indo-1/AM dye by HSC-3 cells were dose-dependent (Table II).

Table I. *Flow cytometric analysis of reactive oxygen species in human oral squamous cell carcinoma cells (HSC-3) after treatment with various concentrations of berberine.*

Berberine (μM)	Uptake of DCFH-DA (% control)
0	0.6±0.1
5	13.1±2.1***
10	28.5±3.2***
25	52.8±4.6***
50	79.3±5.8***
75	90.5±8.4***

Values are mean±S.D. n=3. The HSC-3 cells ($5x10^5$ cells/ml) were treated with 0, 5, 10, 25, 50 and 75 μM berberine. The zero concentration was defined as the control. The percentage of cells taking up the DCFH-DA dye was determined by flow cytometry as described in the Materials and Methods section. ***Significantly different from the control at $p<0.01$.

Table II. *Flow cytometric analysis of Ca^{2+} concentration in human oral squamous cell carcinoma cells (HSC-3) with treatment of various concentrations of berberine.*

Berberine (μM)	Uptake of Indo-1/AM (% control)
0	1.9±0.4
5	11.2±1.8***
10	22.2±2.7***
25	48.6±5.2***
50	71.4±7.0***
75	86.2±7.9***

Values are mean ± S.D. n=3. The HSC-3 cells ($5x10^5$ cells/ml) were treated with 0, 5, 10, 25, 50 and 75 μM berberine. The zero concentration was defined as the control. The percentage of cells taking up the Indo-1/AM dye was determined by flow cytometry as described in the Materials and Methods section. ***Significantly different from the control at $p<0.01$.

Effects of berberine on the mitochondria membrane potential from HSC-3 cells. The mitochondria membrane potential (MMP) was significantly lower in the berberine-treated than in control groups. The effects of berberine on the levels of MMP, determined by the uptake of the $DiOC_6$ dye by HSC-3 cells, were dose-dependent (Table III).

Table III. *Flow cytometric analysis of mitochondrial membrane potential in human oral squamous cell carcinoma cells (HSC-3) with treatment of various concentrations of berberine.*

Berberine (μM)	Percentage of cells taking up $DiOC_6$
0 (control)	90.8 ± 8.4
5	69.8 ± 7.3***
10	54.2±4.8***
25	31.2±4.1***
50	21.8±1.9***
75	12.6±1.8***

Values are mean ± S.D. n=3. The HSC-3 cells ($5x10^5$ cells/ml) were treated with 0, 5, 10, 25, 50 and 75 μM berberine. The zero concentration was defined as the control. The percentage of cells taking up the $DiOC_6$ dye was determined by flow cytometry as described in the Materials and Methods section. ***Significantly different from the control at $p<0.01$.

Inhibition of berberine-induced caspase-3 activity and apoptosis by the caspase inhibitor z-VAD-fmk in HSC-3 cells. The data show that berberine increased caspase-3 activity and these effects were dose-dependent (Figure 3A). The HSC-3 cells were pretreated with the cell-permeable broad-spectrum caspase inhibitor z-VAD-fmk 3 h prior to the treatment with berberine. z-VAD-fmk reduced caspase-3 activity (Figure 3B). After treatment of HSC-3 cells with berberine and z-VAD-fmk, inhibition of berberine-mediated caspase-3 activation was accompanied by the marked attenuation of berberine-induced apoptotic cell death (Figure 3C).

Western blotting for examining the effect of berberine on p53, BAX, Bcl-2 and cytochrome c expression of HSC-3 cells. The results indicated that berberine increased the expression of p53 (Figure 4A), phosphorylation (Figure 4B) and cytochrome *c* release (Figure 4A), but reduced the expression of Bcl-2 (Figure 4C).

Discussion

It is well-known that an inappropriate expression of pro-apoptotic and anti-apoptotic associated proteins results in uncontrolled behaviors of proliferation and death of cells which can be triggered by anticancer agents. The best strategy for chemical anticancer agents is to induce apoptosis of cancer cells. However, the mechanism by which agents induce apoptosis occurs through different pathways, which are still not completely understood and include activation of either caspase-dependent or caspase-independent pathways (20).

Our previous studies had shown that berberine induced apoptosis in HL-60 cells through mitochondrial- and caspase-dependent pathways (18). On the other hand, berberine-induced mitochondrial disruption is involved in both ROS generation and cleavage of BID, a Bcl-2 family member (18). Berberine was originally obtained from the Chinese herb *Rhizoma coptidis* (10) and used as a Chinese herbal medicine (10). Although berberine is reported to inhibit cell growth and induce apoptosis of various cancer cells (14-17), there is no evidence of its pharmacological efficacy on oral cancer cells. Berberine, as well as lovastatin, an inhibitor of the mevalonate

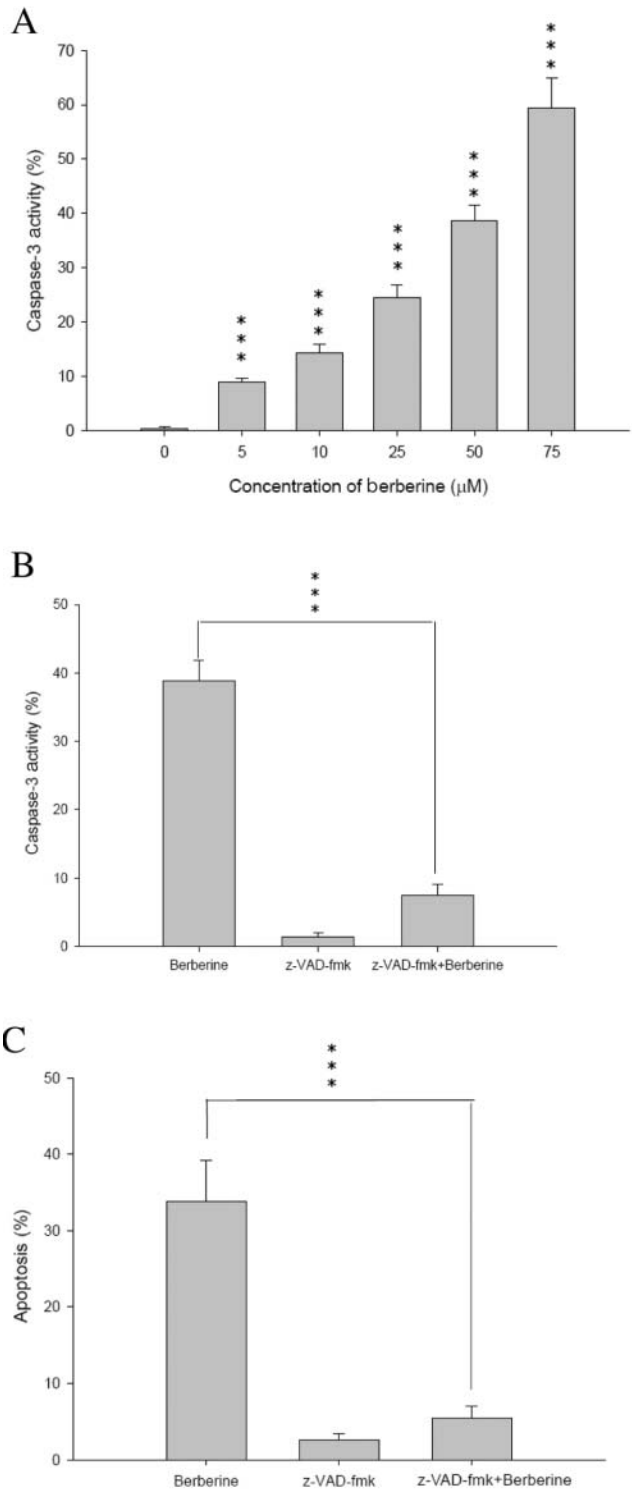

Figure 3. *Flow cytometric analysis of the effects of berberine on caspase-3 activity and apoptosis in HSC-3 cells. The HSC-3 cells were incubated with various concentrations of berberine or 100 μM berberine with or without z-VAD-fmk treatment prior to caspase-3 activity (panel A and B) and apoptosis determination (panel C) as described in Materials and Methods. Data represent mean±S.D. of three experiments. Significantly different from the control was at *p<0.05 and ***p<0.01.*

pathway, exerted dose-dependent cytostatic/cytotoxic effects against human breast cancer cells (MDA-MB231) (21). Sudheer *et al.* demonstrated that berberine induced apoptosis in human epidermoid carcinoma A431 cells, which was associated with an increased expression of pro-apoptotic protein BAX, reduced expression of anti-apoptotic proteins Bcl-2 and Bcl-xL, disruption of the mitochondrial membrane potential, and activation of caspase-9 and -3, and poly ADP-ribose polymerase (22).

In the present study, we demonstrated that berberine induced apoptotic death of HSC-3 oral cancer cells in a dose- and time-dependent manner. Treatment with berberine resulted in a decrease in Bcl-2 expression and an increase in BAX expression, promoting caspase-3 activation, and inducing mitochondrial dysfunction (reduction of the levels of MMP). Therefore, pre-treatment with a caspase inhibitor (z-VAD-fmk) led to a reduction of the caspase-3 activity caused by berberine and also reduced the percentage of apoptosis in HSC-3 cells.

In order to gain mechanical insights into berberine-induced apoptosis, the catalytic activity of caspase-3 and mitochondrial dysfunction in HSC-3 cells were investigated. The data revealed that berberine induced ROS and Ca^{2+} production in HSC-3 cells and these effects were dose-dependent. Berberine also promoted the FAS and FADD levels in HSC-3 cells based on the results shown in Figure 4. The effect of berberine on the MMP was closely-correlated with cell apoptosis, which indicates that berberine-induced cell apoptosis was most likely due to its effectin the mitochondria. Berberine-induced cell death revealed phenotypic characteristics of apoptosis, including nuclear fragmentation and accumulation of cells in the sub-G0/G1 fraction from flow cytometric analysis (data not shown). Our data from Western blotting also demonstrated that there were increased levels of cytochrome *c* in the cytosol from berberine-treated cells compared to the control group. We herein demonstrated that berberine increased the enzymatic activity of caspase-3. Furthermore, berberine also resulted in mitochondrial dysfunction evidenced by loss of MMP, changes in expression of Bcl-2 and BAX proteins and cytosolic release of cytochrome *c*. It has been reported that mitochondria are important regulators of apoptosis (23). The major apoptotic characteristics of cells are mitochondrial swelling and disruptions of the outer mitochondrial membrane (24).

In conclusion, we demonstrated that berberine induced apoptosis in human oral cancer HSC-3 cells through the production of ROS and Ca^{2+}, an increase of BAX and a decrease of Bcl-2 which led to the changes of MMP resulting in cytochrome *c* and caspase-9 release followed by the activation of caspase-3, thus causing apoptosis as summarized in Figure 5. Taken together, our data suggest that berberine may be useful in the treatment of oral cancer.

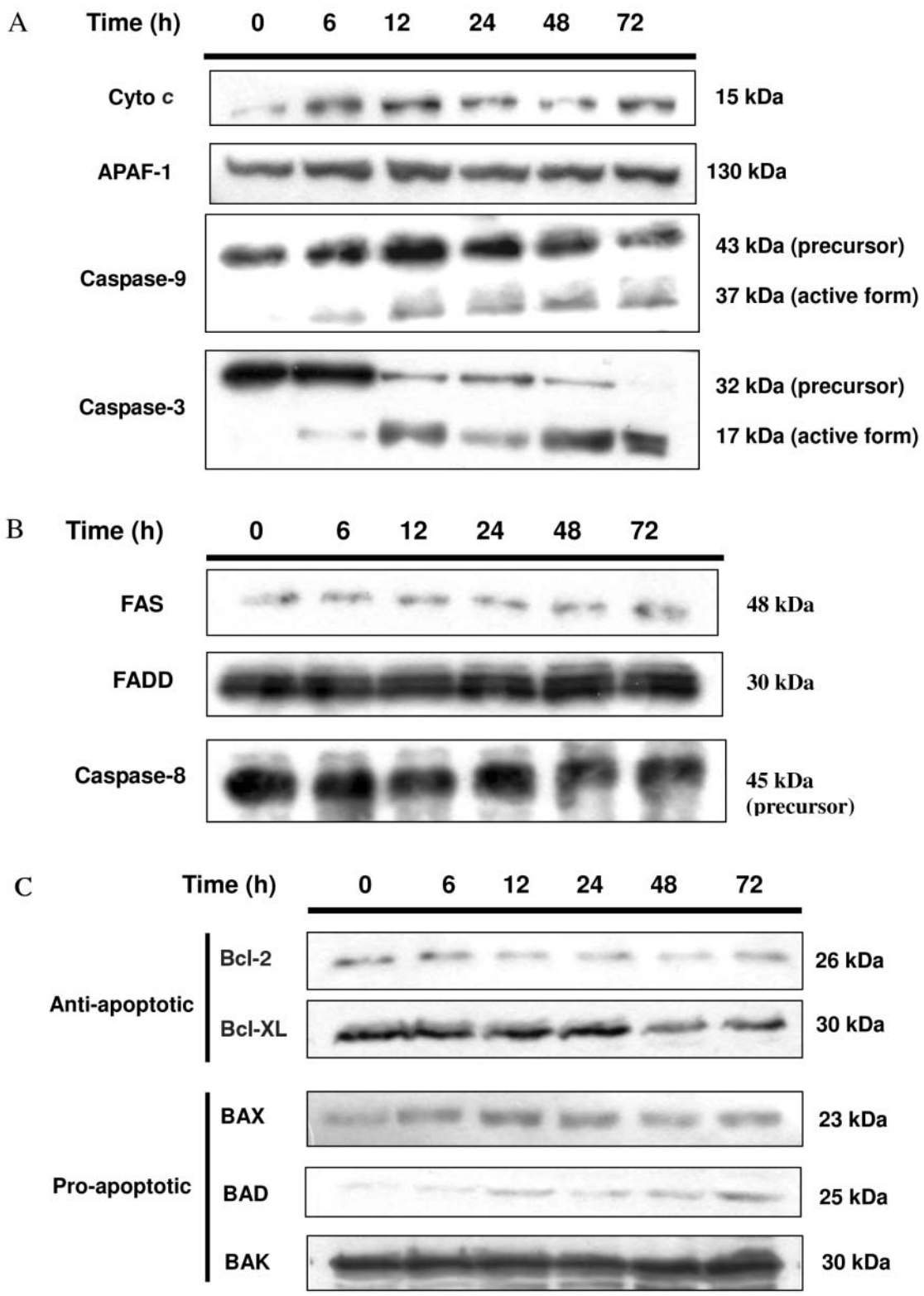

Figure 4. *Representative Western blot showing changes in the levels of cytochrome c, APAF-1, caspase-9,-3, FAS, FADD, caspase-8, Bcl-2, Bcl-xL, BAX, BAD and BAK in HSC-3 cells after exposure to berberine. HSC-3 cells (5x106/ml) were treated with 50 μM berberine for 0, 6, 12, 24, 48 and 72 hours before cytosolic fraction and total protein were prepared and determined as described in Materials and Methods. Cytochrome c, APAF-1, caspase-9, caspase-3, FAS, FADD, caspase-8, Bcl-2, Bcl-xL, BAX, BAD and BAK expressions were estimated by Western blotting as described in Materials and Methods.*

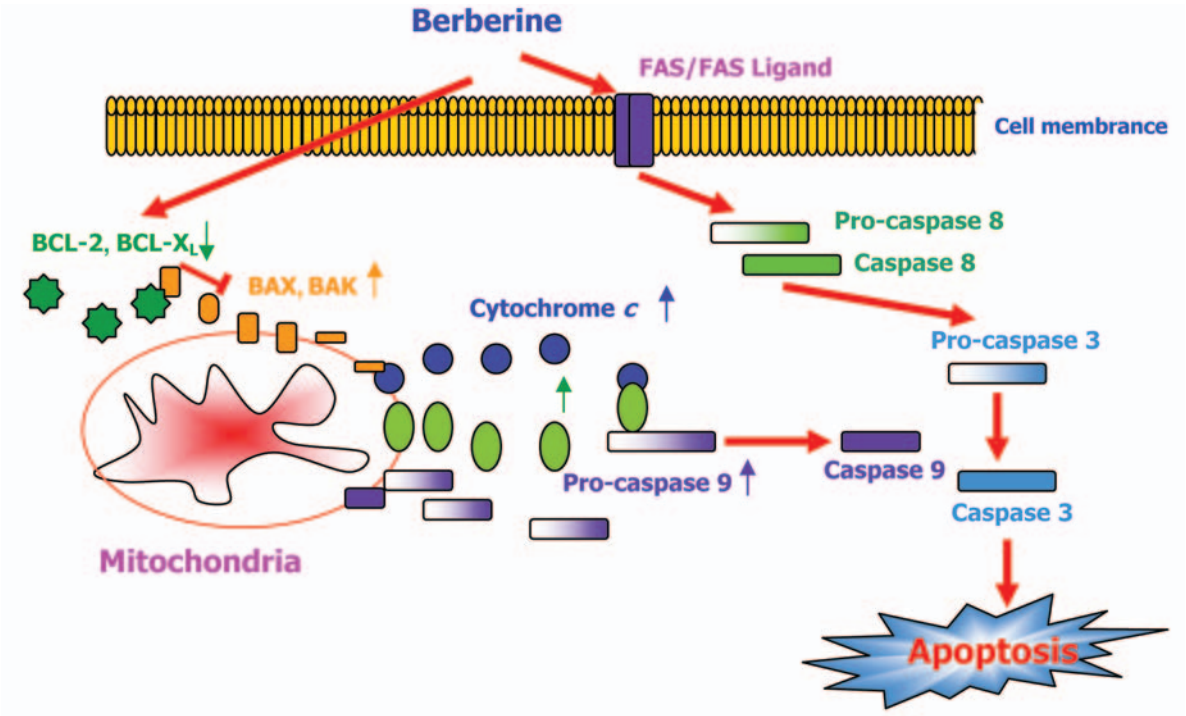

Figure 5. *Proposed model of the mechanism of action of berberine for apoptosis in HSC-3 cells. Berberine induced apoptosis in human oral cancer HSC-3 cells through the production of ROS and Ca^{2+}, an increase of BAX and then decreased Bcl-2 which led to the decrease of MMP leading to cytochrome c and caspase-9 release followed by the activation of caspase-3, thus causing apoptosis.*

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References

1 Parkin DM, Pisani P and Ferlay J: Estimates of the worldwide incidence of 25 major cancers. Int J Cancer *80*: 827-841, 1999.
2 Blo WJ, Devesa SS, McLaughlin JK and Fraumeni JF Jr: Oral and pharyngeal cancers. Cancer Surv *19-20*: 23-42, 1994.
3 Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS and Preston-Martin S,Bernstein L, Schoenberg JB, Stemhagen A and Fraumeni JF, Joseph F: Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res *48*: 3282-3287, 1988.
4 Franco EL, Kowalski LP, Oliveira BV, Curado MP, Pereira RN, Silva ME, Fava AS and Torloni H: Risk factors for oral cancer in Brazil: a case-control study. Int J Cancer *43*: 992-000, 1998.
5 Schlecht NF, Franco EL, Pintos J, Negassa A, Kowalski LP and Oliveira BV *et al*: Interaction between tobacco and alcohol consumption and the risk of cancers of the upper aero-digestive tract in Brazil. Am J Epidemiol *150*: 1129-1137, 1999.
6 McLaughlin JK, Gridley G, Block G, Winn DM, Preston-Martin S and Schoenberg JB, Greenberg RS, Stemhagen A, Austin DF, Ershow AG, Blot WJ and Fraumeni JF Jr: Dietary factors in oral and pharyngeal cancer. J Nat Cancer Inst *80*: 1237-1243, 1988.
7 Franceschi S, Bidoli E, Barón AE, Barra S, Talamini R, Serraino D and La Vecchia C: Nutrition and cancer of the oral cavity and pharynx in north-east Italy. Int J Cancer *47*: 20-25, 1991.
8 Velly AM, Franco EL, Schlecht N, Pintos J, Kowalski LP, Oliveira BV and Curado MP: Relationship between dental factors and risk of upper aerodigestive tract cancer. Oral Oncol *34*: 284-291, 1998.
9 Pintos J, Franco EL, Kowalski LP, Oliveira BV and Curado MP: Use of wood stoves and risk of cancers of the upper aero-digestive tract: a case-control study. Int J Epidemiol *27*: 936-940, 1998.
10 Leng SH, Lu FE and Xu LJ: Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. Acta Pharmacol Sin *25*: 496-502, 2004.
11 Lau CW, Yao XQ and Chen ZY, Ko WH and Huang Y: Cardiovascular actions of berberine. Cardiovasc Drug Rev *19*: 234-244, 2001.
12 Chen QM and Xie MZ: Studies on the hypoglycemic effect of *Coptis chinensis* and berberine. Yao Xue Xue Bao *21*: 401-406, 1986.
13 Gao CR, Zhang JQ and Huang QL: Experimental study on berberin raised insulin sensitivity in insulin resistance rat models. Zhongguo Zhong Xi Yi Jie He Za Zhi *17*: 162-164, 1997.

14 Lin JP, Yang JS, Lee JH, Hsieh WT and Chung J-G: Berberine induces cell cycle arrest and apoptosis in human gastric carcinoma SNU-5 cell line. World J Gastroenterol *12*: 21-28, 2006.
15 Lin CC, Kao ST, Chen GW, Ho HC and Chung J-G: 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(1A)*: 227-242, 2006.
16 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(10)*: 2018-2027, 2006
17 Jantova S, Cipak L and Letasiova S: Berberine induces apoptosis through a mitochondrial/caspase pathway in human promonocytic U937 cells. Toxicol In Vitro *21(1):* 25-32, 2007.
18 Lin CC, Kao ST, Chen GW, Ho HC and Chung J-G : 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(1A)*: 227-242, 2006.
19 Kuo HM, Chang LS, Lin YL, Lu HF, Yang JS, Lee JH and Chung J-G: Morin inhibits the growth of human leukemia HL-60 cells *via* cell cycle arrest and induction of apoptosis through mitochondria-dependent pathway. Anticancer Res *27(1A)*: 395-406, 2007.
20 Rosato RR and Grant S: Histone deacetylase inhibitors in cancer therapy. Cancer Biol Ther *2*: 30-37, 2003.
21 Issat T, Jakobisiak M and Golab J: Berberine, a natural cholesterol reducing product, exerts antitumor cytostatic/cytotoxic effects independently from the mevalonate pathway. Oncol Rep *16(6)*: 1273-1276, 2006.
22 Sudheer KM, Som DS and Santosh KK: 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(10)*: 2018-2027, 2006.
23 Green DR and Reed JC: Mitochondria and apoptosis. Science *281*: 1309-1312, 1998.
24 Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT and Thomson CB: Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria. Cell *91*: 627-637, 1997.

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