Berberine, a Natural Product, Combined with Cisplatin Enhanced Apoptosis through a Mitochondria/Caspase-Mediated Pathway in HeLa Cells

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Berberine, a main component of Coptidis Rhizoma, has been extensively studied and is known to exhibit multiple pharmacologic activities. In this study, we investigated whether the combination of berberine and cisplatin exhibited significant cytotoxicity in HeLa cells. Apoptosis was evaluated based on DNA fragmentation and cytfluorometrically with the annexin-V/propidium iodide labeling method. Combined treatment with berberine and cisplatin acted in concert to induce loss of mitochondrial membrane potential (∆Ψm), release of cytochrome- c from mitochondria, and decreased expression of antiapoptotic Bcl-2, Bcl-xL, resulting in activation of caspases and apoptosis. Further study showed that cell death induced by the combined treatment was associated with increased reactive oxygen species generation and lipid peroxidation. Moreover, we discovered that the combined treatment-induced apoptosis was mediated by the activation of the caspase cascade. These results indicated that the potential of cytotoxicity mediated through the mitochondria-caspase pathway is primarily involved in the combined treatment-induced apoptosis.

Key words berberine; cisplatin; combined treatment; apoptosis; mitochondria-caspase pathway

One of the primary issues in cancer chemotherapy is how to kill cancer cells without damaging normal cells. Clinically, however, the toxicity of anticancer drugs can cause damage to normal cells, resulting in severe side effects, and thus limits their clinical efficacy. To treat cancers with fewer side effects, oncologists have empirically developed many protocols of combination chemotherapy.

Cisplatin and its derivatives are effective agents in cancer therapy. They have been widely used to treat a variety of tumor types including testicular, ovarian, head, and cervical carcinoma.1–3) However, the administration of cisplatin is associated with serious side effects, including nephrotoxic, ototoxic, and neurotoxic events. Frequently, cisplatin is administered in combination with other drugs.4–6) The combined use of two or more chemotherapy agents is often advantageous as it may permit lowering of drug dosages and consequently decrease cytotoxicity, reducing the opportunity for the development of drug resistance by cancer cells.7,8) Several studies aimed at finding drugs able to potentiate the anticancer effect of cisplatin without increasing the serious side effects but were not been successful.

Berberine is an isquinoline derivative alkaloid isolated from Chinese medicinal herbs, such as Hydrastis Canadensis (goldenseal), Cortex phellodendri (Huangbai), and Rhizoma coptidis (Huanglian). Berberine, the major ingredient of these herbs, has many pharmacologic effects including inhibition of DNA and protein synthesis, arrest of cell cycle progress, and possesses anticancer effects9,10) and it is relatively nontoxic to humans.11) Recently, it has been reported that berberine has dual topoisomerase I and II poisoning activity and binds to double helical DNA with high affinity.

Many cancer chemotherapeutic agents act by causing cells to undergo apoptosis. Apoptosis is a physiologic mode of cell death, which can be selectively triggered by cells in response to various stimuli.12) Mitochondria also are important regulators of apoptosis and undergo major changes during apoptotic cell death induced by apoptotic stimuli.13) Early in the induction of apoptosis, a loss of mitochondrial membrane potential can be detected. This leads to the release of cytochrome-c into the cytoplasm, resulting in activation of caspases-3 following activation of caspase-9.14) Antiapoptotic members of the Bcl-2 family, such as Bcl-2, and Bcl-xL, can prevent these mitochondrial events, whereas proapoptotic Bcl-2 family members can trigger them. In addition, mitochondrial production of reactive oxygen species (ROS) seems to play a role in cell death.15,16) ROS, which are the by-products of normal cellular oxidative processes, have been suggested to regulate the process involved in the initiation of apoptotic signaling. Oxidative stress occurs in cells when the generation of ROS overwhelms their natural antioxidant defense system. There is a growing consensus that oxidative stress and the redox state of a cell play a pivotal role in regulating apoptosis.

In the present study, we investigated whether berberine in combination with cisplatin exerts significantly cytotoxic effects against human cervical cancer HeLa cells and examined possible mechanisms of action of the combination.

MATERIALS AND METHODS

Cell Culture and Reagents The human Hela cell line was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) and maintained in RPMI-1640 medium (Gibco, Grand Island, NY, U.S.A.) supplemented with heat-inactivated fetal bovine serum (Gibco), penicillin (100 U/ml), and streptomycin (100 μg/ml) under 5% CO2 in

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a humidified incubator at 37°C. Cisplatin (CDDP) and berberine were purchased from Sigma (St. Louis, MO, U.S.A.), Rhodamin 123, JC-1, dichlorofluorescein diacetate (DCF-DA), and hydroethidium (HE) were purchased from Molecular Probes (Eugene, OR, U.S.A.). Caspase inhibitors (z-DEVD-FMK, z-LEDH-FMK) were purchased from CalbioChem (San Diego, CA, U.S.A.). Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). All other chemicals used were of the highest grade available commercially.

**MTT Assay** Cell viability was quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, the cells were plated in 48-well plates at the density of 5×10^4 cells/well and allowed to adhere at 37°C for 12 h. The following day, various doses of berberine or cisplatin were added and the cells incubated for 24 h, after which cell growth and viability were measured using the MTT assay. The ability of cells to form formazan crystals by active mitochondrial respiration was determined using a microplate reader (Titertek Multiskan, Flow Laboratories, North Ryde, Austria) after dissolving the crystals in DMSO. Cytotoxicity was expressed as the percentage of the absorbance measured in the control cells.

**Apoptosis Assay** To quantify the apoptotic cells, the occurrence of apoptosis was determined by staining cells with both annexin V-FITC (PharMingen, San Diego, CA, U.S.A.) and propidium iodide (PI; Sigma); annexin V can identify externalization of phosphatidylserine during the early apoptotic progression and detect cells in the early stages of the apoptotic process. Briefly, cells were treated with berberine and cisplatin for 17 h. The cells were washed with cold PBS and then resuspended in binding buffer (HEPES/NaOH 10 mM, pH 7.4, NaCl 140 mM, CaCl_2 2.5 mM) at a concentration of 1×10^6 cells/ml. Five microliters of annexin V-FITC and 20 µl of PI were added to these cells, which were then analyzed using a FACSCalibur instrument (BD Bioscience, U.S.A.) equipped with CellQuest®Pro software. The early apoptotic cells stained with annexin V, which show green fluorescence, are represented in the lower right quadrant of the dot plot, and the late apoptotic cells stained with annexin V and PI, which show red-green fluorescence, are represented in the upper right quadrant of the dot plot.

To prepare genomic DNA, cells were incubated with berberine, cisplatin, or their combination for 24 h, then the cells were detached and the cell suspension was centrifuged at 100×g for 10 min. Genomic DNA from the cells was isolated using a DNA isolation kit (Promega, Basel, Switzerland) according to the manufacturer’s instructions. The DNA samples were analyzed on a 1.5% agarose gel containing ethidium bromide (1 µg/ml) in TBE buffer (Tris 100 mM, boric acid 90 mM, EDTA 1 mM). After electrophoresis, the DNA was visualized under UV light and photographed.

**Measurement of Mitochondrial Membrane Potential** Concurrent determinations of mitochondrial inner membrane potential (ΔΨm) dissipation were assayed using Rhodamine 123 staining in accordance with the procedure described by Huigsloot et al. Briefly, cells in 6-cm tissue culture plates were treated with berberine or cisplatin alone or in combination for the indicated times. Twenty minutes before the cells were harvested, Rhodamine 123 was added directly to the culture medium to a final concentration of 30 nM. The cells were harvested by trypsinization, washed with 5 ml of PBS at 37°C, collected by centrifugation, resuspended in 500 ml of using fluorescence-activated cell-sorting analysis (FACS) buffer, and analyzed immediately for Rhodamine 123 fluorescence intensity using flow cytometry (FACSCalibur, BD Bioscience). Additionally, ΔΨm depolarization was detected using the dye JC-1 under a fluorescent microscope at an original magnification ×200. JC-1 selectively accumulates within intact mitochondria to form multimer J-aggregates emitting fluorescent light at 590 nm. The monomeric form emits light at 527 nm after excitation at 490 nm. Thus the color of the dye changes from orange to green, depending on the mitochondrial membrane potential.

**Western Blot Analysis** Cytosolic protein extracts was prepared as previously described. Briefly, cells were collected by centrifugation at 300×g for 5 min at 4°C and washed with ice-cold PBS. The cell pellet was then resuspended in 500 µl of lysis buffer (Hepes-KOH 20 mM, pH 7.5, sucrose 210 mM, mannitol 70 mM, MgCl_2 1.5 mM, KCl 10 mM, leupeptin 10 µg/ml, and digitonin 10 µg/ml). After 10 min incubation at 25°C, the sample was spun at 14000×g for 15 min, and the supernatant containing cytosolic proteins was stored at −70°C until analysis by polyacrylamide gel electrophoresis (SDS-PAGE). The protein extract was subjected to standard SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Millipore, U.S.A.), and probed with appropriate antibodies as described individually in the figure legends. The bound primary antibody was detected using appropriate horseradish peroxidase-conjugated secondary antibody, and protein was visualized using an enhanced chemiluminescence detection kit. Western blot analysis was done using primary antibodies against cytochrome-c, VDAC, pro-caspases 3 and 9, Bcl-2, Bax, Bcl-x/L, and β-actin (Santa Cruz Biotechnology) at optimal dilution. β-Actin was used as an internal control to confirm that the amounts of protein load were equal.

**Detection of Cytochrome-c Release** Cytochrome-c released from mitochondria was determined with western blot analysis. Cytosolic and mitochondrial fractions were prepared as below, but the fractionation buffer contained sucrose 250 mM, KCl 10 mM, MgCl_2 1.5 mM, dithiothreitol 1 mM, EDTA 1 mM, EGTA 1 mM, HEPES 20 mM, pH 7.0, and protease inhibitors (sodium orthovanadate 1 mM, leupeptin 10 mg/ml, aprotinin 10 mg/ml, and phenylmethylsulfonyl fluoride 1 mM). The mitochondrial pellet was resuspended in the same buffer, sonicated, and centrifuged to obtain the soluble fraction. Equal amounts of protein were separated on a 15% SDS polyacrylamide gel and transblotted onto polyvinylidene difluoride-nitrocellulose filters. Specific cytochrome-c bands were detected using anti-cytochrome-c antibody (1:500) followed by enhanced chemiluminescence-based detection.

**Measurement of Intracellular ROS Production** To monitor the intracellular ROS, we utilized two different cell-permeable probes, DCF-DA and hydroethidium (HE). Non-fluorescent DCF-DA, hydrolyzed to DCFH inside cells, yields highly fluorescent DCF-DA in the presence of intracellular hydrogen peroxide and related peroxides. Blue fluorescent dihydroethidium is oxidized to red fluorescent ethidium in the presence of superoxide. Therefore the DCF-DA and ethidium fluorescence intensity is proportional to the
Combined Berberine and Cisplatin Enhanced Cell Death in HeLa Cells To evaluate the effects of berberine in combination with cisplatin on cell viability, we investigated the cytotoxic effects of the combined treatment in HeLa cells. Initial screening was done using the MTT assay with a relative exposure of 24 h to the various concentrations of berberine. These experiments with berberine were done to identify a concentration that produced modest cytotoxicity to test its subsequent combination with cisplatin. As shown in Fig. 1A, berberine inhibited cell viability in a dose-dependent manner. We next evaluated the cytotoxicity of berberine in combination with cisplatin. We found that a dose of berberine 50 μg/ml induced a slight, but significant (17%) cytotoxic effect on HeLa cells. However, berberine significantly enhanced cytotoxicity (54%) when combined with cisplatin 5 μM (Fig. 1B). Hence berberine 50 μg/ml and cisplatin 5 μM were selected as the doses used for the combination effects in HeLa cells. We next examined the effects of combined treatment with berberine and cisplatin on HeLa cells in a time-dependent manner (Fig. 1C). The combined treatment showed enhanced cytotoxicity in a time-dependent manner.
observed with berberine or cisplatin alone (Fig. 2B). Taken together, these data indicate that the combination of berberine and cisplatin result in significant apoptotic death in HeLa cells.

Combined Berberine and Cisplatin Induced Mitochondrial Dysfunction of HeLa Cells
Since disruption of the mitochondrial membrane potential (MMP) is a critical step in cells undergoing apoptosis, we evaluated whether combined treatment with berberine and cisplatin had any effect on the MMP using Rhodamine 123 as a marker of mitochondrial membrane integrity. Disruption of the MMP caused a decrease in FL-1 fluorescence as analyzed with flow cytometry.

Fig. 2. Combined Berberine and Cisplatin Induced Apoptotic Cell Death
Cells were treated with berberine (50 μg/ml) and/or cisplatin (5 μM) for 24 h. (A) The fraction of cells undergoing apoptotic cell death was detected using annexin V + PI staining. a) Control; b) berberine (50 μg/ml); c) cisplatin (5 μM); d) berberine and cisplatin. (B) Detection of DNA ladder formation. (C) Quantification of cells undergoing apoptosis. Data are mean±S.D. of three independent experiments. **p<0.01 compared with control group.

Fig. 3. Combined Berberine and Cisplatin Exhibited Cytotoxicity through the Mitochondria-Mediated Apoptosis Pathway
The change in mitochondrial membrane potential (ΔΨm) in HeLa cells treated with berberine (50 μg/ml) and cisplatin (5 μM). (A) Representative histograms showing ΔΨm of cells. The cells were stained with Rhodamine 123, then analyzed with flow cytometry. (B) JC-1 detected by fluorescent microscopy. a) Control; b) berberine (50 μg/ml); c) cisplatin (5 μM); d) berberine and cisplatin. (C) Effects of combined berberine and cisplatin on mitochondrial cytochrome-c release in HeLa cells. (D) Western blot analysis of expression of Bcl-2 family in HeLa cells treated with berberine (50 μg/ml) and cisplatin (5 μM). Scale bar, 100 μm. BB, berberine; Cis, cisplatin; C.F., cytoplasmic fraction; M.F., mitochondrial fraction; VDAC, voltage-dependent anion-selective channel protein; Cyto.c, cytochrome-c.
try. Figure 3A shows no substantial change in the MMP after treatment with berberine 50 \(\mu g/ml\) or cisplatin 5 \(\mu M\) alone. However, when the cells were treated with berberine and cisplatin in combination, markedly decreased Rhodamine 123 fluorescence was observed, indicating disruption of the MMP in these cells. In fluorescent microscopy images, mitochondrial membrane depolarizations were detected due to the lack of orange fluorescence of the J-aggregate at hyperpolarized membrane potential (Fig. 3B). This was in accordance with the results of Rhodamine 123 staining (Fig. 3A).

One of the consequences of MMP disruption is the release of cytochrome-c into the cytosol.\(^{22}\) While cytochrome-c protein levels in the cytosolic fractions of cells treated with berberine or cisplatin alone for 24 h showed no substantial increase, the protein levels were increased in cells treated with combined berberine and cisplatin (Fig. 3C). The release of mitochondrial cytochrome-c can directly be induced by proapoptotic members of the Bcl-2 family member Bax.\(^{31}\) To clarify the mechanism of combined treatment-induced apoptosis in HeLa cells, some Bcl-2 family proteins were detected after the cells were treated for 24 h. The results revealed that the expression of Bcl-2 and Bcl-XL was downregulated while the expression of Bax was upregulated concurrently after combined treatment with berberine and cisplatin in HeLa cells (Fig. 3D).

**Combined Berberine and Cisplatin Increased Intracellular ROS Generation and Lipid Peroxidation** Because mitochondrial respiratory chain on the inner mitochondrial membrane is a major intracellular source of ROS,\(^{29}\) we next examined whether berberine in combination with cisplatin induces ROS generation. First, we examined the level of ROS production after combined treatment with berberine and cisplatin using DCF-DA, a ROS-sensitive probe, in HeLa cells. In contrast to the control or berberine or cisplatin alone, treatment with berberine 50 \(\mu g/ml\) and cisplatin 5 \(\mu M\) induced intense fluorescence in HeLa cells (Fig. 4A). We further monitored superoxide anion (O\(_2^-\)) generation by HE, which selectively detects O\(_2^-\). At 18 h, there was a distinct increase of ROS generation in combined-treatment cells compared with that in cells treated with either berberine or cisplatin alone (Figs. 4A, B), suggesting that combined treatment may maintain ROS at higher levels. These findings suggest that the combination of berberine and cisplatin promotes ROS generation to levels necessary for cell death.

We finally addressed the effects of combined berberine and cisplatin treatment on the content of intracellular lipid peroxidation products (TBARs). We observed that TBAR (lipid peroxidation) formation in the cells treated with berberine or cisplatin alone showed no significant differences (Fig. 4C). However, combined treatment of the cells with berberine and cisplatin significantly increased TBAR formation (2-fold). As shown in Fig. 4C, berberine in combination with cisplatin increased TBAR formation.

**Combined Treatment-Induced Apoptosis Requires Activation of Caspases in HeLa Cells** As the family of aspartate-specific cysteinyl proteases (caspases) plays a pivotal role in the execution of programmed cell death,\(^{25}\) we determined whether the induction of apoptosis by the combination of berberine and cisplatin resulted in activation of upstream caspase-9 and downstream caspase-3 (Fig. 5). The activation of caspases was measured using fluorogenic analysis and Western blot analysis, respectively, in HeLa cells treated with berberine or cisplatin alone and their combination. As shown in Figs. 5A and C, HeLa cells after combined with berberine and cisplatin showed increased caspase-9 activity (2.5-fold) and procaspase-9 clearly cleaved forms of caspase-9. Caspase-9 plays a role mainly in the mitochondria-mediated pathway. Moreover, we analyzed the activation of caspase-3 because it has been shown to be one of the most important cell executioners in apoptosis. The combined treatment with berberine and cisplatin increased in the activation of caspase-3 (2.3-fold) and procaspase-3 clearly cleaved forms of caspase-3 as analyzed by Western blotting (Figs. 5B, C), whereas the activation of these caspases was not detected in cells treated with berberine or cisplatin alone. Next, to test whether caspase activation was required for combined treatment-induced apoptosis, we used a potent inhibitor of caspases, z-DEVD-FMK (a caspase-3 inhibitor) and z-LEDH-FMK (a caspase-9 inhibitor). Cells pretreated with 100 \(\mu M\) of each of the above caspase inhibitors were further incubated for 24 h with berberine or cisplatin alone or combined. As shown in Fig. 5D, combined treatment with berberine and cisplatin resulted in 58.2% apoptosis compared with control.
cells, whereas the combined treatment in the presence of the pan-caspase inhibitor (z-DEVD-FMK) resulted in only 10% cell death. Thus the presence of the pan-caspase inhibitor significantly blocked combined treatment-induced apoptosis in HeLa cells. Taken together, the results indicate that combined treatment-induced apoptosis in HeLa cells is mediated primarily through the activation of caspases.

DISCUSSION

In the present study, we investigated the combined effects of berberine and cisplatin on human cervical cancer HeLa cells. Our initial experiments investigating the effects of combined treatment on cell viability showed an enhanced cytotoxic effect on Hela cells with relatively low doses of berberine and cisplatin. We then investigated the molecular and biochemical pathway involved in combined treatment-induced apoptosis in the hope of providing an experimental basis for the clinical application of this combination.

Apoptosis is a process of gene-mediated programmed cell death essential for the elimination of unwanted cells in various biological systems and is the key mechanism of chemotherapeutic agents. The mode of drug-induced apoptosis has also been described at the level of modifying the intracellular oxidative state. Interestingly, mitochondria was both the source and target of ROS. ROS are predominantly produced in the mitochondria, lead to the free radical attack of membrane phospholipids and loss of MMP, which caused the intermembrane proteins, such as cytochrome-c, to be released from the mitochondria and ultimately triggered caspase-3 activation. Moreover, the changes in cellular redox potential due to enhanced generation of ROS and increase in lipid peroxidation are sufficient to induce apoptosis.

Berberine belongs to the camptothecin family of drugs characterized by the ability to induce DNA topoisomerase poisoning and hence apoptotic cell death. Recent studies have shown that berberine induced inhibition of cell growth and apoptosis in various cell lines. Hwang et al. reported that berberine induces apoptosis through a mitochondria/caspase pathway in human hepatoma cells. In this work, we have shown that the combination of berberine and cisplatin was able to enhance cytotoxicity in HeLa cells. As far as we know, there is no report indicating the combined effects with berberine and cisplatin in HeLa cells. Our findings demonstrate that combined treatment with berberine and cisplatin clearly induced apoptotic cell death of HeLa cells showing DNA laddering, and annexin V/PI staining (Fig. 2). Bcl-2 proteins may be one of the key factors in the common final pathways involved in the regulation of apoptosis. Moreover, the combination treatment resulted in loss of the MMP and downregulation of Bcl-2. Proapoptotic members of the Bcl-2 family play important roles in drug-induced apoptosis by control of mitochondrial permeability due to their ability to form channels in membranes and to regulate preexisting channels. In this study, our results revealed that combined treatment-induced apoptosis was related to upregulation of Bax as well as downregulation of Bcl-2 and Bcl-XL (Fig. 3). Therefore we suggest that combined treatment-induced apoptosis in HeLa cells was caused by regulation of mitochondrial permeability. The actions of ROS are secondary to the breakdown of \( \Delta \Psi_m \), and it has been suggested that the response of mitochondria to ROS could affect drug cytotoxicity. Permeabilization of the mitochondrial membrane and collapse of the \( \Delta \Psi_m \) can cause increased ROS generation from mitochondria. ROS, such as hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), hydroxyl radicals (\( \text{OH}^- \)), superoxide anion
radicals (O$_3$), and lipid peroxidation, are known mediators of intracellular signaling cascades.\textsuperscript{31)} Mitochondrial permeabilization and activation of caspases have been reported in cell death induced by berberine or cisplatin.\textsuperscript{12,28)} These results are consistent with the studies that suggested the involvement of ROS and mitochondrial potential transit in berberine-induced apoptotic stress.\textsuperscript{28,32,33)} In our study, the results in Fig. 4 indicate that combined berberine and cisplatin induced intracellular oxidation, as measured by the formation of oxidized DCF-DA and HE. It is noted that berberine-induced O$_2^-$ generation was slightly greater than cisplatin-induced O$_2^-$ generation. These results suggest that the level of ROS species was slightly different after berberine- or cisplatin-induced ROS production. Another study showed that the berberine induced superoxide anion radical in SW620 human colonic carcinoma cells.\textsuperscript{33)} Also, Hirakawa \textit{et al.}\textsuperscript{33)} reported that berberine can induce O$_2^-$ species formation when complexed with DNA molecules. In this study, we had no direct evidence to verify the mechanism of ROS generation between berberine and cisplatin alone. However, we will perform a further study to address this question.

Some of the caspase activation involved in berberine- or cisplatin-induced apoptosis were investigated in various cell lines.\textsuperscript{26,34)} In particular, caspase-3 has been shown to be a key component of the apoptotic machinery. We found that combined treatment with berberine and cisplatin induced apoptosis by activation of the downstream caspase-3, which has been shown to play an important role in apoptosis induced by several conditions. In our results, caspase-3 activation was preceded by the activation of caspase-9, the apical caspase of the intrinsic mitochondrial pathway of apoptosis (at the 3 h). On the other hand, activation of caspase-8, the apical caspase of the extrinsic pathway, became evident only later (data not shown). This result suggests that caspase-9 may play the main role in the initial triggering of the cleavage, and activation of procaspase-3 may represent a downstream event after the activation of caspase-9. The primary involvement of the caspase activation in combined treatment-induced apoptosis was confirmed by observation that specific inhibitors of caspases inhibited combined treatment-induced apoptosis in HeLa cells.

In summary, our results demonstrate that the combined application of berberine (50 μg/ml) with a low dose of cisplatin (5 μM) resulted in a statistically significant decrease in cell survival in comparison with berberine or cisplatin treatment alone. Therefore cytotoxic potential of berberine can be amplified by a combination with cisplatin. Based on the results obtained in this study, we hypothesized a mode of action in which combined with berberine and cisplatin caused depletion of ∆ψ\textsubscript{m} soon after cellular uptake with suppression of Bcl-2. The ∆ψ\textsubscript{m} depletion facilitated the release of cytochrome-c from mitochondria. Subsequently, cytochrome-c activated the subsequent caspases and caused DNA fragmentation. Oxidative stress also was likely to affect the cytotoxicity of combined treatment by regulating ∆ψ\textsubscript{m}. Based on these results, our study clearly points out that there is an increased effect on growth inhibition in the cervical cancer cell line used. On the basis of these promising results further investigations and \textit{in vivo} trials must be performed to determine the possible benefits of clinical applications.

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**REFERENCES**