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Antitumor Effects of Camptothecin Combined with Conventional Anticancer Drugs on the Cervical and Uterine Squamous Cell Carcinoma Cell Line SiHa

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Functional defects in mitochondria are involved in the induction of cell death in cancer cells. We assessed the toxic effect of camptothecin against the human cervical and uterine tumor cell line SiHa with respect to the mitochondria-mediated cell death process, and examined the combined effect of camptothecin and anticancer drugs. Camptothecin caused apoptosis in SiHa cells by inducing mitochondrial membrane permeability changes that lead to the loss of mitochondrial membrane potential, decreased Bcl-2 levels, cytochrome *c* release, caspase-3 activation, formation of reactive oxygen species and depletion of GSH. Combination of camptothecin with other anticancer drugs (carboplatin, paclitaxel, doxorubicin and mitomycin c) or signaling inhibitors (farnesyltransferase inhibitor and ERK inhibitor) did not enhance the camptothecin-induced cell death and caspase-3 activation. These results suggest that camptothecin may cause cell death in SiHa cells by inducing changes in mitochondrial membrane permeability, which leads to cytochrome *c* release and activation of caspase-3. This effect is also associated with increased formation of reactive oxygen species and depletion of GSH. Combination with other anticancer drugs (or signaling inhibitors) does not appear to increase the anti-tumor effect of camptothecin against SiHa cells, but rather may reduce it. Combination of camptothecin with other anticancer drugs does not seem to provide a benefit in the treatment of cervical and uterine cancer compared with camptothecin monotherapy.

Key Words: **Camptothecin, SiHa cells, Mitochondrial membrane permeability change, Cell death, Combined effect of anticancer drugs**

INTRODUCTION

Mitochondria are considered a cellular target for anticancer drugs. The mitochondrial therapeutic approach against cancer uses strategies that either modulate the action of Bcl-2 family members at the mitochondrial outer membrane or induce formation of the mitochondrial permeability transition (Armstrong, 2006). The mitochondrial permeability transition formation has been shown to cause depolarization of the transmembrane potential, release of Ca^{2+} and cytochrome *c*, and activation of caspases, which results in cell death (Mignotte and Vayssière, 1998; Hong et al., 2003; Lee et al., 2004).

Topoisomerase I is essential for DNA replication (Porter and Champoux, 1989; Nitiss and Wang, 1996). Camptothecin and its analogs stabilize the DNA-topoisomerase I cleavable complex and cause accumulation of single-stranded breaks in DNA. The collision of a DNA replication fork with the cleaved strand of DNA causes an irreversible double-strand DNA break, ultimately leading to cell death (Tsao et al., 1993; Shao et al., 1999). Camptothecin and its analogs, such as topotecan, are used in the therapy of ovarian, cervical, colorectal and small cell lung cancers (Arbuck and Takimoto, 1998; Ackermann et al., 2007).

Camptothecin analogs exhibit toxic effects against cancer cells by inducing the activation of apoptotic caspases and formation of ROS (Wenzel et al., 2004; Xia et al., 2005). Glioblastoma cells exposed to camptothecin exhibit activation of caspase-3, -8, and -9 (Xia et al., 2005). Meanwhile, topotecan induces cell death in non-small cell lung cancer cells in a caspase-8-dependent manner without caspase-9 activation (Ferreira et al., 2000). Camptothecin and its analogs are suggested to exhibit their toxic effects by increasing ROS formation. However, the cytotoxic effect is attenuated by thiol compounds glutathione and *N*-acetylcysteine (Timur et al., 2005; Xia et al., 2005; Huang et al., 2006). Therefore, the mechanism of the anticancer effect of camptothecin remains controversial. Topoisomerase I inhibitor causes apoptotic cell death in cancer cells by inducing caspase activation, cell surface Fas receptor activation, and ROS formation (Xia et al., 2005). In contrast, the anticancer

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ABBREVIATIONS: FTI, farnesyltransferase inhibitor; ERKi, extracellular signal-regulated kinase inhibitor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $DiOC_6(3)$, 3,3'-dihexyloxacarbocyanine iodide; $DCFH_2$-DA, dichlorofluorescin diacetate; DTNB, 5,5'-dithio-bis-(2-nitrobenzoic acid); PMSF, phenylmethylsulfonylfluoride; ROS, reactive oxygen species.

drug cisplatin and the topoisomerase II inhibitor etoposide induce apoptosis without cell surface death receptor activation (Wang et al., 2006). Furthermore, it is uncertain whether mitomycin c-induced cell death is mediated by caspase-3 activation (Kobayashi et al., 2000; Pirnia et al., 2002). The signal transduction pathways involved in cellular differentiation, growth and death are considered therapeutic targets for anticancer drugs (Faivre et al., 2006). Nevertheless, the combined effect of signaling inhibition on camptothecin toxicity remains uncertain.

Defects in mitochondrial function are involved in the induction of cancer cell death. Nevertheless, it has not been clarified whether the actions of anticancer drugs involve the mitochondria-mediated process. Combined treatment with anticancer drugs may exert a synergistic toxic effect against tumor cells while causing less damage to normal cells. We first investigated the toxic effect of camptothecin against the human cervical and uterine tumor cell line SiHa with respect to the mitochondria-mediated cell death process, and then assessed the combined effect of other anticancer drugs (or signaling inhibitors) on camptothecin toxicity.

METHODS

Materials

Quantikine® immunoassay kits for human cytochrome *c* and caspase-3 were purchased from R&D Systems (Minneapolis, MN, USA); anti Bcl-2 and anti-cytochrome *c* (A-8) antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); farnesyltransferase inhibitor (FTI; type II, H-Cys-4-Abz-Met-OH), extracellular signal-regulated kinase (ERK) activation inhibitor peptide (type I, Ste-MEK1$_{13}$), carboplatin, paclitaxel and horseradish peroxidase-conjugated anti-mouse IgG were from EMD- Calbiochem. Co. (La Jolla, CA, USA); and SuperSignal® West Pico chemiluminescence substrate was from PIERCE Biotechnology Inc. (Rockford, IL, USA). Camptothecin, doxorubicin, mitomycin c, MTT, DiOC$_6$(3), monoclonal β-actin antibody, DCFH$_2$-DA, DTNB, PMSF and other chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Cell culture

SiHa cells (origin: human cervix and uterus; histopathology: squamous cell carcinoma) were obtained from Korean cell line bank (Seoul, South Korea). SiHa cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml of penicillin and 100 μg/ml of streptomycin in a 5% CO_2 atmosphere at 37°C as described in the manual of the cell line bank. Twenty-four hours before the experiments, cells were washed with DMEM containing 1% FBS and replated onto 96-, 48- or 24-well plates.

Cell viability assay

Cell viability was measured by the MTT assay, which is based on the conversion of MTT to formazan by mitochondrial dehydrogenases (Mosmann, 1983). SiHa cells (2×10^4 cells/200 μl) were treated with various concentrations of camptothecin for 24 h at 37°C and then incubated with 10 μl of 10 mg/ml MTT solution for 2 h. After centrifugation at 412×g for 10 min, the culture medium was removed and 100 μl of dimethyl sulfoxide was added to each well to dissolve the formazan. Absorbance was measured at 570 nm using a microplate reader (Spectra MAX 340, Molecular Devices Co., Sunnyvale, CA, USA). Cell viability was expressed as a percentage of the absorbance in control cultures.

Measurement of Bcl-2 and cytochrome c

The levels of Bcl-2 and cytochrome *c* were assessed by western blot analyses and solid-phase enzyme-linked immunosorbent assays. SiHa cells (5×10^6 cells/2.5 ml for western blotting and 5×10^5 cells/ml for ELISA) were harvested by centrifugation at 412×g for 10 min, washed twice with PBS, resuspended in buffer (in mM) [sucrose 250, KCl 10, $MgCl_2$ 1.5, EDTA 1, EGTA 1, dithiothreitol 0.5, PMSF 0.1 and HEPES-KOH 20, pH 7.5], and homogenized by successive passages through a 26-gauge hypodermic needle. The homogenates were centrifuged at 100,000×g for 30 min and the supernatant was used for western blot analysis and ELISA assay. Protein concentration was determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA).

For western blotting, supernatants were mixed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and boiled for 5 min. Samples (30 μg protein/well) were loaded onto each lane of 15% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (GE Healthcare Chalfont St. Giles, Buckinghamshire, UK). Membranes were blocked for 2 h in TBS (50 mM Tris-HCl, pH 7.5 and 150 mM NaCl) containing 0.1% Tween 20 and 5% non-fat dried milk. The membranes were labeled with primary antibodies (anti-mouse Bcl-2, anti-cytochrome *c* and β-actin) overnight at 4°C with gentle agitation. After four washes in TBS containing 0.1% Tween 20, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG for 2 h at room temperature. Membranes were treated with SuperSignal® West Pico chemiluminescence substrate and the proteins were identified by detecting the enhanced chemiluminescence in the Luminescent image analyzer (Lite for Las-1000 plus version 1.1, Fuji Photo Film Co., Tokyo, Japan).

For the ELISA-based quantitative analysis, supernatants and cytochrome *c* conjugate were added to 96-well microplates coated with monoclonal antibody specific for human cytochrome *c*. The procedure was performed according to the manufacturer's instructions. Sample absorbance was measured at 450 nm in a microplate reader. A standard curve was constructed using diluted cytochrome *c* standard. The amount was expressed as ng/ml by reference to the standard curve.

Measurement of caspase-3 activity

SiHa cells (2×10^6 cells/ml) were treated with camptothecin for 24 h at 37°C and caspase-3 activity was determined with ApoAlert™ CPP32/caspase-3 assay kit. The supernatant obtained by centrifugation of lysed cells was added to the reaction mixture containing dithiothreitol and caspase-3 substrate (*N*-acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide) and incubated for 1 h at 37°C. Absorbance of the chromophore *p*-nitroanilide was measured at 405 nm. A standard curve was obtained from the absorbance of *p*-nitroanilide standard reagent diluted with cell lysis buffer (up to 20 nM). One unit of enzyme was defined as the activity producing 1 nmol of *p*-nitroanilide.

Measurement of intracellular ROS formation

The dye $DCFH_2$-DA, which is oxidized to fluorescent dichlorofluorescin (DCF) by hydroperoxides, was used to measure relative levels of cellular peroxides (Fu et al., 1998). SiHa cells (2×10^4 cells/200 μl) were treated with camptothecin for 24 h at 37°C, washed, suspended in FBS-free DMEM, incubated with 50 μM $DCFH_2$-DA for 30 min at 37°C and washed with PBS. The cell suspensions were centrifuged at 412×g for 10 min and medium was removed. Cells were dissolved with 1% Triton X-100 and fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a fluorescence microplate reader (SPECTRAFLUOR, TECAN, Salzburg, Austria).

Measurement of total glutathione

Total glutathione (reduced form GSH+oxidized form glutathione disulfide) was measured using glutathione reductase (van Klaveren et al., 1997). SiHa cells (2×10^4 cells/200 μl) were treated with camptothecin for 24 h at 37°C and centrifuged at 412×g for 10 min in a microplate centrifuge; the medium was then removed. The pellets were washed twice with PBS, dissolved with 2% 5-sulfosalicylic acid (100 μl) and incubated in 100 μl of reaction mixture containing 22 mM sodium EDTA, 600 μM NADPH, 12 mM DTNB and 105 mM NaH_2PO_4, pH 7.5, at 37°C. Glutathione reductase (final concentration, 10 units/ml) was added and the mixture was incubated for 10 min. Absorbance was measured at 412 nm using a microplate reader. A standard curve was obtained by measuring the absorbance of diluted commercial GSH incubated in the same manner as tne samples.

Statistical analysis

Data are expressed as mean±SEM. Statistical analysis was performed by one-way analysis of variance. When significance was detected, post hoc comparisons between groups were made using Duncan's test for multiple comparisons. A probability less than 0.05 was considered statistically significant.

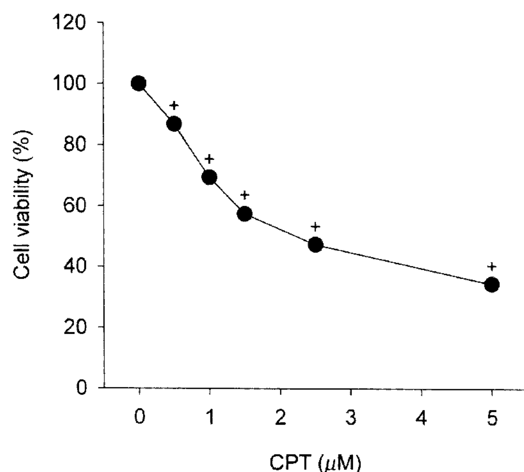


Fig. 1. Camptothecin induces cell viability loss. SiHa cells were treated with either 0.5~5 μM camptothecin (CPT) for 24 h and then cell viability was determined by using the MTT assay. Data represent mean±SEM (n=6). $^+p<0.05$ compared to control (percentage of control).

RESULTS

Camptothecin toxicity against SiHa cells

The predominant cell type in cervical cancer is squamous cell carcinoma (about 80~85%). Thus, the present study examined camptothecin toxicity using the human cervical and uterine tumor cell line SiHa. When SiHa cells were treated with 0.5~5 μM camptothecin, cell viability decreased in a concentration-dependent manner. The incidence of cell death after treatment with 2.5 μM camptothecin for 24 h was about 53% (Fig. 1).

Camptothecin induces mitochondrial membrane permeability changes

To better characterize the cell death signaling events in camptothecin toxicity, we investigated the effect of camptothecin on changes in mitochondrial membrane permeability. Changes in the mitochondrial transmembrane potential in SiHa cells treated with camptothecin were quantified by flow cytometry with the dye $DiOC_6(3)$. Treatment with 2.5~5 μM camptothecin for 24 h increased the percentage of SiHa cells with depolarized mitochondria (characterized by low values of transmembrane potential) (Fig. 2A).

As a mitochondria-mediated cell death signaling event, opening of the mitochondrial permeability transition pore causes the release of cytochrome *c* from mitochondria into the cytosol and subsequent caspases activation (Crow et al., 2004; Kim et al., 2006). Bax induces permeability of the outer mitochondrial membrane and elicits a pro-apoptotic response by stimulating the release of cytochrome *c*; this effect is blocked by Bcl-2. We examined whether camptothecin toxicity was mediated by mitochondrial apoptotic pathway. In western blotting analysis, SiHa cells treated with 2.5~5 μM camptothecin for 24 h exhibited decreased anti-apoptotic Bcl-2 protein levels and increased cytochrome *c* levels (Fig. 2B).

We confirmed the camptothecin-induced change in the mitochondrial membrane permeability, which results in cytochrome *c* release and caspase-3 activation, by performing ELISA-based quantitative analyses. Camptothecin increased the cytochrome *c* release and caspase-3 activity in SiHa cells in a dose-dependent manner (Fig. 2C).

Camptothecin induces formation of ROS and depletion of GSH

To determine whether the cytotoxicity of camptothecin could be ascribed to oxidative stress, we investigated the effect of camptothecin on ROS formation and GSH depletion in SiHa cells. ROS formation within SiHa cells was measured by monitoring conversion of $DCFH_2$-DA to DCF. Treatment with 1~5 μM camptothecin significantly increased DCF fluorescence, and this response was concentration dependent (Fig. 3A). The camptothecin (2.5 μM)-induced increase in DCF fluorescence was prevented by the addition of 1 mM *N*-acetylcysteine.

The involvement of ROS in camptothecin toxicity was further assessed by using various scavengers. Treatment with 1 mM *N*-acetylcysteine (a thiol compound), 30 μM trolox

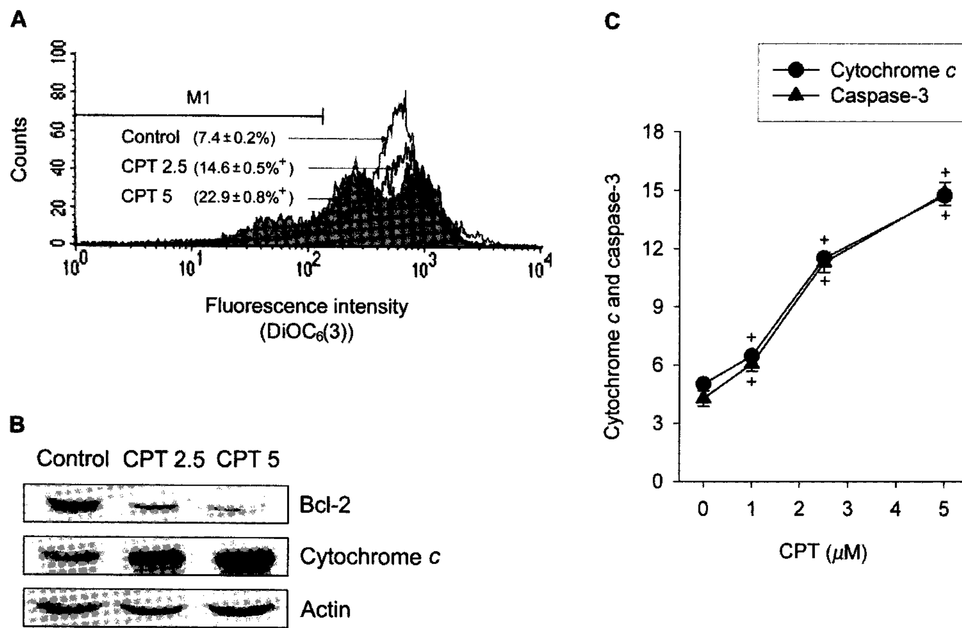


Fig. 2. Camptothecin induces cytochrome c release and caspase-3 activation. SiHa cells were treated with 1~5 μM camptothecin (CPT) for 24 h. In the assay (A) for the mitochondrial transmembrane potential, data are expressed as the percentage of cells with depolarized mitochondria for the mitochondrial membrane potential and represent mean±SEM (n=4). In western blot assay (B), the levels of Bcl-2 and cytochrome c in the cytosolic fractions were analyzed by western blotting with antibodies (anti Bcl-2 and anti-cytochrome c). Data are representative of three different experiments. In ELISA-based quantitative analysis (C), values represent ng/ml for cytochrome c release and arbitrary units (a.u.) for caspase-3 activity. Data are mean±SEM (n=6). $^{+}p<0.05$ compared to control.

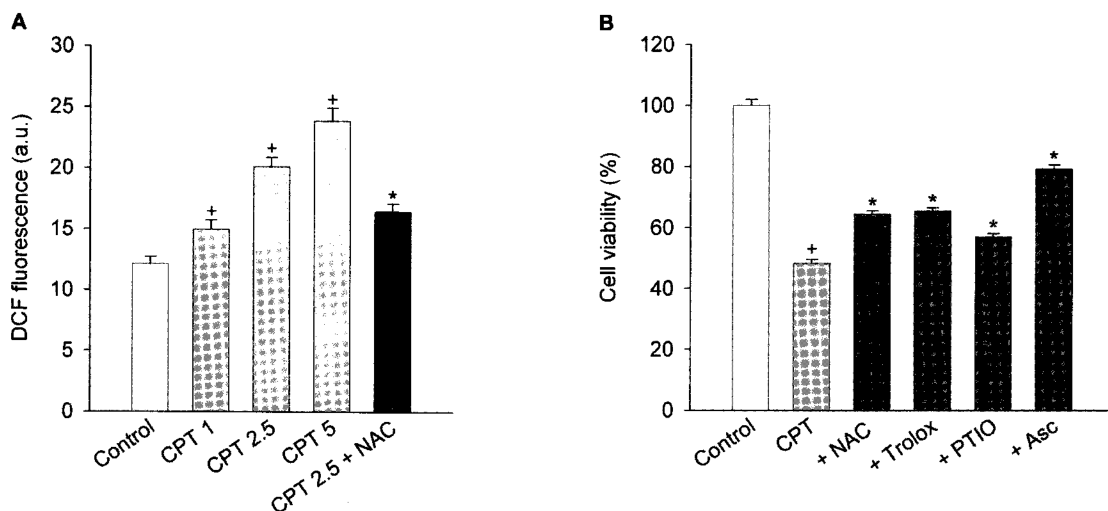


Fig. 3. Camptothecin induces formation of reactive oxygen species. In experiment (A) for reactive oxygen species formation, SiHa cells were treated with either 1~5 μM camptothecin (CPT) or 2.5 μM camptothecin plus 1 mM *N*-acetylcysteine (NAC) for 24 h. Data are expressed as arbitrary units of fluorescence (a.u.) and represent mean±SEM (n=6). $^{+}p<0.05$ compared to control; and $^{*}p<0.05$ compared to camptothecin alone. In experiment (B) for cell death, SiHa cells were treated with 2.5 μM camptothecin (CPT) in combination with scavengers [(1 mM *N*-acetylcysteine (NAC), 30 μM trolox, 30 μM carboxy-PTIO (PTIO) and 1 mM ascorbate (Asc)] for 24 h, and cell viability was determined. Data represent mean±SEM (n=6). $^{+}p<0.05$ compared to control; and $^{*}p<0.05$ compared to camptothecin alone.

(a scavenger of hydroxyl radicals and peroxynitrite), 30 μM carboxy-PTIO (a scavenger of nitric oxide) and 1 mM ascorbate (an inhibitor of lipid peroxidation and oxidant scavenger) inhibited the camptothecin-induced cell death (Fig. 3B).

Reduction of cellular GSH levels increases the sensitivity of neurons to toxic insults and induces changes in mitochondrial function (Hall, 1999). This work assessed whether the cytotoxic effect of camptothecin was ascribed to its depleting effect on cellular GSH content. The thiol content in control SiHa cells was 12.07±0.12 nmol/mg protein. Camptothecin decreased the cellular GSH content in a dose-dependent manner. When cells were treated with 2.5 μM camptothecin for 24 h, GSH content decreased to 52% (Fig. 4).

Combined effect of anticancer drugs on camptothecin toxicity

We examined the combined effect of anticancer drugs on camptothecin toxicity. Treatment with 2.5 μM camptothecin for 24 h induced 52% cell death in SiHa cells. Other anticancer drugs (100 μM carboplatin, 100 μM paclitaxel, 10 μM doxorubicin and 3 μg/ml mitomycin c) caused 11~

49% cell death. Combined treatment with anticancer drugs did not augment camptothecin toxicity. The combined toxic effect of camptothecin and other anticancer drugs (carboplatin, paclitaxel, doxorubicin or mitomycin c) was less than the sum of the effect of each anticancer drug alone (Fig. 5A); this was further confirmed by neutral red uptake (Fig. 5B).

We next investigated the effects of cell signaling inhibitors on camptothecin toxicity. Treatment with 0.5 μM FTI or 5 μM ERK inhibitor for 24 h caused 16~28% cell death. Combination of FTI (or ERK inhibitor) and camptothecin did not increase camptothecin toxicity, but rather reduced the toxic effect (Fig. 6).

To confirm the combined effect of camptothecin and anticancer drugs, we examined the activation of the apoptosis effector caspase-3 in cells. As with cell death, the combination of camptothecin and anticancer drugs (or signaling inhibitors) did not have a synergistic effect on caspase-3 activation in SiHa cells (Fig. 7). The combined toxic effect of camptothecin and other anticancer drugs (or signaling inhibitors) was similar to or less than the sum of the effect each anticancer drug alone.

DISCUSSION

Drugs that affect mitochondrial function may interfere with survival and proliferation of cancer cells (Dias and Bailly, 2005; Armstrong, 2006). Camptothecin analogs, including topotecan, used concurrently with cisplatin may be the new standard of care for the management of recurrent or advanced cervical cancer (Ackermann et al., 2007). Camptothecin analogs have been shown to cause cell death

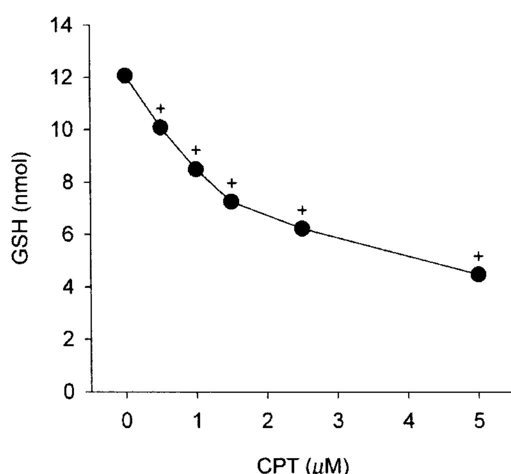


Fig. 4. Camptothecin induces depletion of GSH contents. SiHa cells were treated with 0.5~5 μM camptothecin (CPT) for 24 h. Data are expressed as nmol of GSH/mg protein and represent mean±SEM (n=6). $^{+}p<0.05$ compared to control.

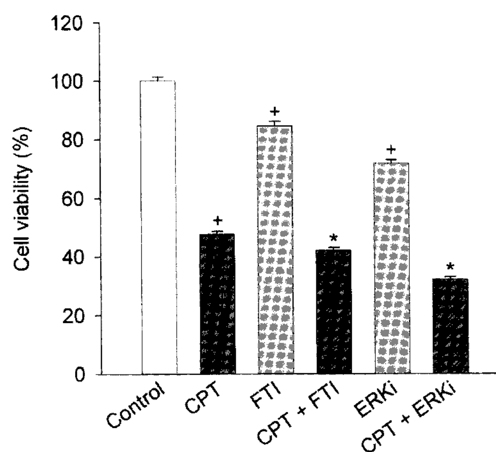
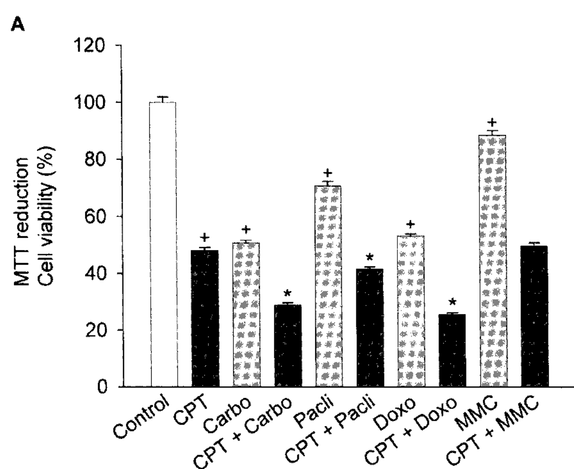


Fig. 6. Effect of signaling inhibitors on camptothecin-induced cell death. SiHa cells were treated with 2.5 μM camptothecin (CPT) in combination with 0.5 μM FTI [or 5 μM ERK inhibitor (ERKi)] for 24 h and then cell viability was measured. Data represent mean±SEM (n=6). $^{+}p<0.05$ compared to control; and $^{*}p<0.05$ compared to camptothecin alone.



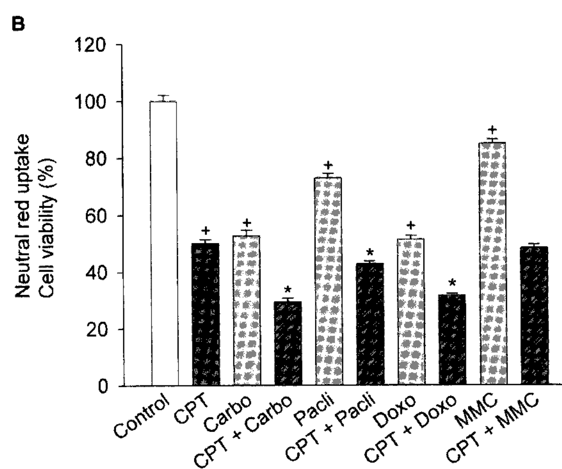


Fig. 5. Effect of anticancer drugs on camptothecin-induced cell death. SiHa cells were treated with 2.5 μM camptothecin (CPT) in combination with anticancer drugs [100 μM carboplatin (Carbo), 100 μM paclitaxel (Pacli), 10 μM doxorubicin (Doxo) or 3 μg/ml mitomycin c (MMC)] for 24 h, and then cell viability was measured by using MTT reduction assay (A) or neutral red uptake assay (B). Data represent mean±SEM (n=6). $^{+}p<0.05$ compared to control; and $^{*}p<0.05$ compared to camptothecin alone.

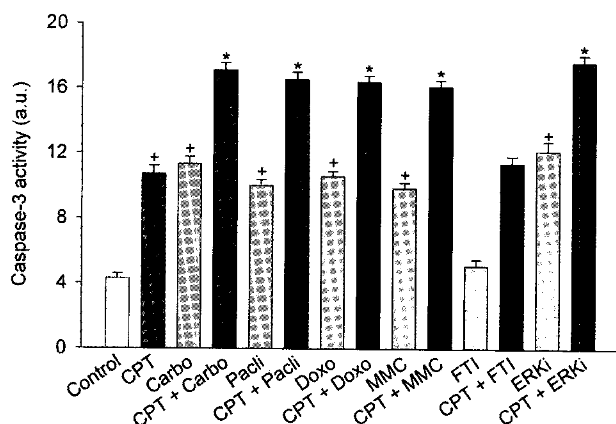


Fig. 7. Effect of anticancer drugs on camptothecin-induced activation of caspase-3. SiHa cells were treated with 2.5 μM camptothecin (CPT) in combination with anticancer drugs [100 μM carboplatin (Carbo), 100 μM paclitaxel (Pacli), 10 μM doxorubicin (Doxo) or 3 μg/ml mitomycin c (MMC)] or signaling inhibitors [0.5 μM and 5 μM ERK inhibitor (ERKi)] for 24 h. Data are expressed as units for caspase-3 activity and represent mean±SEM (n=6). $^+p<0.05$ compared to control; and $^*p<0.05$ compared to camptothecin alone.

in cancer cells by inducing the activation of apoptosis effector caspases (Wenzel et al., 2004; Xia et al., 2005). However, it is uncertain whether camptothecin toxicity against cancer cells is the result of its effects on mitochondria, because camptothecin toxicity is differently mediated by caspases (Ferreira et al., 2000; Xia et al., 2005). In light of the contradictory findings for the mitochondria-mediated cell death process, the toxic effect of camptothecin against the human cervical and uterine tumor cell line SiHa has not been elucidated. One aim of the present study was to assess the toxic effect of camptothecin against SiHa cells in relation to the mitochondria-mediated cell death process and oxidative stress. In the present study, treatment with camptothecin caused significant loss of cell viability in SiHa cells. Opening of the mitochondrial permeability transition pore causes a loss in the transmembrane potential, cytochrome *c* release and caspase-3 activation, which leads to apoptotic cell death (Mignotte and Vayssière, 1998; Lee et al., 2004). The mitochondrial membrane potential loss may induce cytochrome *c* release followed by caspase-3 activation (Armstrong, 2006; Kim et al., 2006). The inhibitory effect of cyclosporin A suggests that camptothecin induces the mitochondrial permeability transition formation, leading to loss of the mitochondrial transmembrane potential. These results suggest that camptothecin induces apoptotic cell death in SiHa by causing the mitochondrial membrane potential loss and by decreasing anti-apoptotic Bcl-2 levels, which results in cytochrome *c* release and subsequent caspase-3 activation.

Camptothecin and its analogs exhibit a toxic effect by inducing ROS formation, which is attenuated by the thiol compounds glutathione and *N*-acetylcysteine (Timur et al., 2005; Xia et al., 2005). However, the toxic effect of topotecan is not attenuated by *N*-acetylcysteine (Huang et al., 2006). Dehydroascorbate, which is rapidly taken up by cells and efficiently reduced to ascorbate, inhibits apoptosis in Jurkat cells treated with camptothecin, but does not affect necrosis (Sane et al., 2004). Because of these contradictory findings, we examined whether the camptothecin-induced formation of the mitochondrial permeability transition was ascribed to oxidative stress. The inhibition of the mitochondrial respiratory chain induced by toxic substances causes the production of ROS and nitrogen species (Brown, 1999; Chandra et al., 2000; Fleury et al., 2002). ROS act upon mitochondria, causing disruption of mitochondrial membrane potential and release of cytochrome *c*. Together with previous reports, the inhibitory effect of antioxidant *N*-acetylcysteine on cell death and the increased formation of ROS suggest that the camptothecin-induced mitochondrial damage and cell death is mediated by increased oxidative stress. Increased production of ROS causes changes in the levels of intracellular antioxidants, such as GSH, NADH or NADPH, which impair mitochondrial function (Constantini et al., 1996). The oxidation and depletion of cellular GSH can modulate opening of the mitochondrial permeability transition pore (Constantini et al., 1996; Hall, 1999). Mitochondrial GSH depletion is suggested to trigger the apoptotic pathway (Hall, 1999). Along with the production of ROS in cells treated with camptothecin, the decrease of cell viability approximately correlated with the effect of camptothecin on GSH depletion. Therefore, the present findings indicate that camptothecin-induced mitochondrial damage and cell death involve increased ROS production and depletion of cellular GSH.

Combining anticancer drugs may provide a beneficial effect in cancer therapy, *i.e.*, a synergistic toxic effect against tumor cells and less damaging effects on normal cells. In this respect, we examined whether combination of camptothecin and conventional anticancer drugs had a synergistic toxic effect. However, as shown in the results, the combination of camptothecin and anticancer drugs (carboplatin, paclitaxel, doxorubicin or mitomycin c) did not exhibit an increased toxic effect against SiHa cells, and rather may have reduced the damaging effect. A similar finding was also observed in SiHa cells co-treated with camptothecin and 18β-glycyrrhetinic acid, a metabolite of glycyrrizin (Lee et al., 2008). They exhibited an additive toxic effect.

The ERK signal transduction pathway regulates cell growth and apoptosis in various cell types (Chang et al., 2003). Topotecan and farnesyltransferase inhibitor cause growth inhibition and induce cell death in human (EA.hy926) endothelial cells and glioblastoma cells by inhibiting ERK expression (Blum et al., 2006; Yang et al., 2007). Nevertheless, the combined effect of camptothecin and cell signaling inhibitors against cancer cells remains uncertain. The present study suggests that inhibition of farnesyltransferase or ERK does not modulate camptothecin toxicity. Therefore, camptothecin monotherapy appears to be more effective in the treatment of cervical and uterine cancer than the combined therapy.

Overall, these results suggest that camptothecin may cause cell death in SiHa cells by inducing the mitochondrial membrane permeability change, which leads to cytochrome *c* release and caspase-3 activation. The effect seems to be associated with increased ROS formation and depletion of GSH. Combining conventional anticancer drugs (or signaling inhibitors) with camptothecin may not increase the anti-tumor effect against SiHa cells. Indeed, combination of camptothecin and other anticancer drugs may not provide a benefit in the treatment of cervical and uterine cancer compared with camptothecin monotherapy.

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