

Sanguinarine Sensitizes Human Gastric Adenocarcinoma AGS Cells to TRAIL-mediated Apoptosis via Down-regulation of AKT and Activation of Caspase-3

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Abstract. Sanguinarine is a benzophenanthridine alkaloid, derived from the root of *Sanguinaria canadensis* and other poppy *Fumaria* species, which is known to have antimicrobial, antiinflammatory and antioxidant properties. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is known to induce apoptosis in cancer cells but spare most normal cells. However, its effects are limited in some types of cancer cells, including AGS human gastric adenocarcinoma cells. In the present study, we showed that treatment with TRAIL in combination with subtoxic concentrations of sanguinarine sensitized TRAIL-mediated apoptosis in AGS cells. Combined treatment with sanguinarine and TRAIL effectively induced Bid cleavage and loss of mitochondrial membrane potential, leading to the activation of caspases, and cleavage of poly(ADP-ribose) polymerase and β -catenin. The cytotoxic effects of the combined treatment were significantly inhibited by α -DEVD-fmk, a caspase-3 inhibitor, which demonstrates the important role of caspase-3 in the observed cytotoxic effect. In addition, the levels of Akt protein were markedly reduced in cells co-treated with sanguinarine and TRAIL. Apoptosis induced by the combined treatment was markedly increased by the phosphatidylinositol-3'-kinase inhibitor, LY294002 (Akt-upstream inhibitor), through the mitochondrial amplification step and caspase activation, suggesting that

interactions of the synergistic effect were at least partially mediated through the Akt-dependent pathway.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a transmembrane protein that is known to engage the extrinsic apoptotic pathway by binding to its membrane-bound death receptors (DR4 and DR5), which transmit an apoptotic signal via their intracellular death domains (1, 2). In several cell lines, cellular sensitivity to TRAIL depends on the expression of cell membrane TRAIL receptors and caspase-8. Caspase-8 is activated in response to TRAIL and released into the cytoplasm, where caspase-8 initiates a protease cascade that activates effector caspases, including caspase-3 and caspase-7 (2, 3).

Sanguinarine (13-methylbenzodi-oxolo[5,6-]1,3-dioxolo[4,5-I] phenanthridinium) is a benzophenanthridine alkaloid that has significant structural homology to chelerythrine, derived from the root of *Sanguinaria canadensis* and other poppy *Fumaria* species (4-6). Sanguinarine has been shown to possess antimicrobial, antioxidant, and antiproliferative properties (7-11). Our previous study suggested that the activity of extracellular signal-regulated kinase (ERK) and Akt was downregulated in sanguinarine-treated cells, and phosphatidylinositol 3'-kinase (PI3K)/Akt inhibitor, LY294002, sensitized the cells to sanguinarine-induced apoptosis, indicating that the downregulation Akt signaling pathway may play a key role in sanguinarine-induced apoptosis (12). However, the exact molecular mechanisms by which these drugs achieve their synergistic effects remain to be fully elucidated.

Recent reports have demonstrated that many tumor cells, including the AGS human gastric adenocarcinoma cell line, acquire resistance to the apoptotic effects of TRAIL (13, 14).

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Mutations of a pro-apoptotic protein, BAX, and increased expression of IAP family members such as XIAP and cIAP can contribute to the resistance to TRAIL-mediated apoptosis (1, 13). However, our previous data suggest that certain natural compounds reversed apoptosis resistance to TRAIL via caspase-3 activation in AGS cells (14) or through down-regulation of AKT and ERK in A549 cells (15). A recent study also showed co-treatment with sanguinarine and TRAIL resulted in reduced levels of BCL-2, which is known to be regulated by reactive oxygen species in MDA-MB-231 cells (16). These results suggest that sanguinarine can be used in combination with TRAIL to sensitize resistant cells to TRAIL-mediated apoptosis.

In the present study, we investigated whether treatment with a combination of TRAIL and sanguinarine may be a safe and effective strategy by which to treat TRAIL-resistant carcinoma. We used a TRAIL-resistant gastric adenocarcinoma AGS cell line as a model system with which to investigate the sensitizing effects of sanguinarine on TRAIL-mediated apoptosis.

Materials and Methods

Materials. Sanguinarine was obtained from Sigma (St Louis, MO, USA). The chemical was dissolved in methanol as a stock solution at 10 mM concentration. Dilutions were made in the culture medium, and it was stored at -20°C . The methanol concentration in the medium was $<0.08\%$ and did not affect cell viability. TRAIL was purchased from KOMA Biotech (Seoul, S. Korea), dissolved in distilled water as a stock solution at 100 $\mu\text{g}/\text{ml}$ and stored at -70°C , dilutions were made in the culture medium. An enhanced chemiluminescence (ECL) kit was purchased from Amersham (Arlington Heights, IL, USA). The caspase-3 inhibitor, z-DEVD-fmk, and the phosphatidylinositol-3'-kinase (PI3K) inhibitor, LY294002, were obtained from Calbiochem (San Diego, CA, USA). All other chemicals not specifically cited here were purchased from Sigma.

Antibodies. Antibodies against BCL-2, BAX, BID, BCL-XL, poly(ADP-ribose) polymerase (PARP), β -catenin, caspase-3, caspase-8, caspase-9 and AKT were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-pAKT antibody was obtained from Cell Signaling Inc. (Danvers, MA, USA). The antibody against actin was purchased from Sigma. Peroxidase-labeled donkey anti-rabbit and sheep anti-mouse immunoglobulin were purchased from Santa Cruz Biotechnology.

Cell culture. Human gastric adenocarcinoma AGS cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured at 37°C in a 5% CO_2 humidified incubator, and maintained in RPMI-1640 culture medium containing 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, Gaithersburg, MD, USA) and 1% penicillin-streptomycin (Gibco BRL).

Cell viability. The cells were seeded 1×10^6 cells/ml in a 100-mm Petri dish and treated with 0-0.8 μM sanguinarine, 100 ng/ml TRAIL, or combined treatment (sanguinarine and TRAIL) for 24

h. Following treatment, cell viability was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Agarose gel electrophoresis for DNA fragmentation assay. The cells were treated with sanguinarine and/or TRAIL for 24 h and lysed in a buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, and 0.5% Triton[®] X-100 for 30 min on ice. The lysates were vortexed and cleared by centrifugation at 14,000 rpm for 25 min. The DNA in the supernatant was extracted using a 25:24:1 (v/v/v) equal volume of neutral phenol:chloroform:isoamyl alcohol and analyzed electrophoretically on 1.5% agarose gels containing 0.1 $\mu\text{g}/\text{ml}$ ethidium bromide (EtBr).

Cytotoxicity assay. The lactate dehydrogenase (LDH) released into cell cultures is an index of cytotoxicity and evaluates the permeability of the cell membrane. After an incubation of 24 h of the combined treatment with sanguinarine and TRAIL, or each alone, the culture media were collected. The LDH assay was performed using the Cytotoxicity Detection kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The percentage of LDH released was expressed as the proportion of the LDH released into the culture medium compared to the total amount of LDH present in cells treated with 2% Triton[®] X-100.

DAPI staining. After treatment with sanguinarine, TRAIL, or combined treatment (sanguinarine and TRAIL) for 24 h, the cells were harvested and washed with phosphate-buffered saline (PBS) and fixed with 3.7% paraformaldehyde in PBS for 10 min at room temperature (RT). The cells were washed with PBS, stained with a solution of 4',6-diamidino-2-phenylindole (DAPI) for 10 min at RT and then washed with distilled water. Finally, the cells were treated with mounting solution on a glass slide, covered with a cover glass, and analyzed via a fluorescent microscope (Carl Zeiss, Germany).

Flow cytometric analysis. The cells were treated with sanguinarine and/or TRAIL for 24 h and harvested by centrifuge at 2,000 rpm for 5 min. After centrifuging, the supernatant was removed, and then the cells were washed in 1 ml PBS. Cell cycle analysis was detected by a DNA reagent kit (Becton Dickinson, San Jose, CA, USA). The cell pellet was combined with 125 μl solution A for 10 min at room temperature, after which 100 μl of solution B were added for 10 min at room temperature, and then 100 μl of solution C was added for 30 min at 4°C in the dark. The stained cells were analyzed for DNA content using a DNA flow cytometer (Becton Dickinson), and CellQuest software was used to determine the relative DNA content based on the presence of red fluorescence.

Protein extraction and Western blot analysis. The cells were lysed in an extraction buffer [25 mM Tris-Cl (pH 7.5), 250 mM NaCl, 5 mM ethylenediaminetetra acetic acid, 1% nonidet P40], 1 mM phenylmethylsulfonyl fluoride and 5 mM dithiothreitol for 30 min. Protein concentration was determined with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). For Western blot analysis, proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then were electrotransferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH, USA). The membranes were subjected to immunoblot analysis with the desired antibodies, and the proteins were visualized by the ECL method (Amersham).

Mitochondrial membrane potential (MMP) assay. To measure the MMP, the dual-emission fluorescent dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide (JC-1) was used. JC-1 is internalized and concentrated by respiring mitochondria and can reflect changes in MMP in living cells. There are two excitation wavelengths: 527 nm (green) for the monomer form and 590 nm (red) for the JC-1 aggregate form. Briefly, the cells were collected and incubated with 10 μ M JC-1 for 30 min at 37°C. The cells were subsequently washed once with cold PBS and analyzed using a DNA flow cytometer.

Determination of caspase activity. The activities of caspase-3, -8, and -9 were determined by colorimetric assay kits from R&D Systems (Minneapolis, MN, USA) following the manufacturer's protocol. Briefly, the cells were lysed in a lysis buffer for 30 min in an ice bath. The lysed cells were centrifuged at 14,000 rpm for 10 min, and 100 μ g of the protein were incubated with 50 μ l of the colorimetric tetrapeptides, Asp-Glu-Val-Asp (DEAD)-*p*-nitroaniline (pNA) for caspase-3, Ile-Glu-Thr-Asp (IETD)-pNA for caspase-8, and Leu-Glu-His-Asp (LEHD)-pNA for caspase-9, respectively, at 37°C for 3 h. The reactions were measured by changes in absorbance at 405 nm using an ELISA reader (Molecular Devices).

Statistical analysis. All data are presented as mean \pm SD. Significant differences among the groups were determined using the unpaired Student's *t*-test. A value of **p*<0.05 was accepted as an indication of statistical significance. All the figures shown in this article were obtained from at least three independent experiments.

Results

Nontoxic dose of sanguinarine significantly triggers TRAIL-mediated apoptosis. To investigate the effects of sanguinarine, TRAIL, or their combined treatment (sanguinarine + TRAIL) on cell viability, AGS cells were treated with the indicated agents and subjected to MTT assays, DNA fragmentation, DAPI staining, and cell cycle analyses. As shown in Figure 1A, treatment with sanguinarine and TRAIL alone for 24 h resulted in only a slight decrease in cell viability (to 94 \pm 5% by sanguinarine and 93 \pm 4% by TRAIL, respectively). We also evaluated the combination of sanguinarine and TRAIL in the AGS cells given our finding that sanguinarine can decrease cell viability in AGS cells. Notably, AGS cells treated with a combination of TRAIL and different concentrations of sanguinarine significantly reduced cell viability more than treatment with TRAIL or sanguinarine alone. Treating cells with TRAIL and 0.4 μ M and 0.8 μ M of sanguinarine reduced cell viability to 61 \pm 5% and 38 \pm 8% of control levels at 24 h, respectively. When co-administered, TRAIL and sanguinarine significantly increased DNA fragmentation and the number of apoptotic bodies relative to sanguinarine or TRAIL alone (Figure 1B and C). To obtain a quantitative measure of apoptosis induction, we next investigated the number of cells with sub-G₁ DNA content using flow cytometric analysis. The combined treatment resulted in a significant accumulation of cells with sub-G₁ DNA content

(21 \pm 6%), whereas treatment with sanguinarine or TRAIL alone did not (Figure 1D). These results suggest that sanguinarine significantly stimulates TRAIL-mediated apoptosis in TRAIL-resistant AGS cells.

Combined treatment with sanguinarine and TRAIL up-regulates truncated BID, and induces loss of MMP, caspase activity, and subsequent cleavage of PARP and β -catenin. Mitochondria appear to play a central role in apoptosis, and have been a major focus of recent studies (17, 18). During apoptotic cell death, the early events that occur are mitochondrial depolarization and loss of cytochrome *c* from the mitochondrial intermembrane space. The pro-apoptotic and anti-apoptotic members of the BAX and BCL-2 proteins play pivotal roles in the regulation of apoptosis. These proteins interpret a wide array of diverse upstream survival and distress signals to determine the fates of the cells (19, 20). Therefore, we investigated whether combined treatment with sanguinarine and TRAIL induces apoptosis by modulating the expression of BCL-2 family members. As shown in Figure 2A, treatment with sanguinarine alone did not affect the expression levels of anti-apoptotic or pro-apoptotic proteins. In contrast, expression of apoptotic BAX was up-regulated, while the apoptotic protein, BID, was truncated by combined treatment. In addition, combined treatment with sanguinarine and TRAIL caused a significant loss of MMP (Figure 2B) but not in TRAIL or sanguinarine alone. Analysis of our data indicates that combined treatment with sanguinarine and TRAIL may increase loss of MMP, and may lead to apoptosis in TRAIL-resistant AGS cells. Caspases are also known to act as important mediators of apoptosis and contribute to the overall apoptotic morphology by cleavage of various cellular substrates (21, 22). Therefore, we investigated the cleavage of caspase-3, -8 and -9, and the subsequent proteolytic cleavage of PARP and β -catenin in AGS cells treated with sanguinarine and TRAIL for 24 h. As shown in Figure 3A, Western blot analysis revealed that treatment with TRAIL alone only slightly affected the cleavage of caspases, PARP, and β -catenin. However, combined treatment with TRAIL and sanguinarine significantly induced the cleavage of caspases, PARP and β -catenin. Cell lysates containing equal amounts of total protein from cells treated with sanguinarine and TRAIL were then assayed for *in vitro* caspase activity. As shown in Figure 3B, combined treatment with TRAIL and sanguinarine significantly increased caspase-3, -8, and -9 activities. These results indicate that combined treatment induces apoptotic death in AGS cells, at least in part through a caspase-dependent pathway.

Inhibition of caspase-3 activity restores cell death following combined treatment with sanguinarine and TRAIL. Caspase-3 represents one of the key proteases responsible

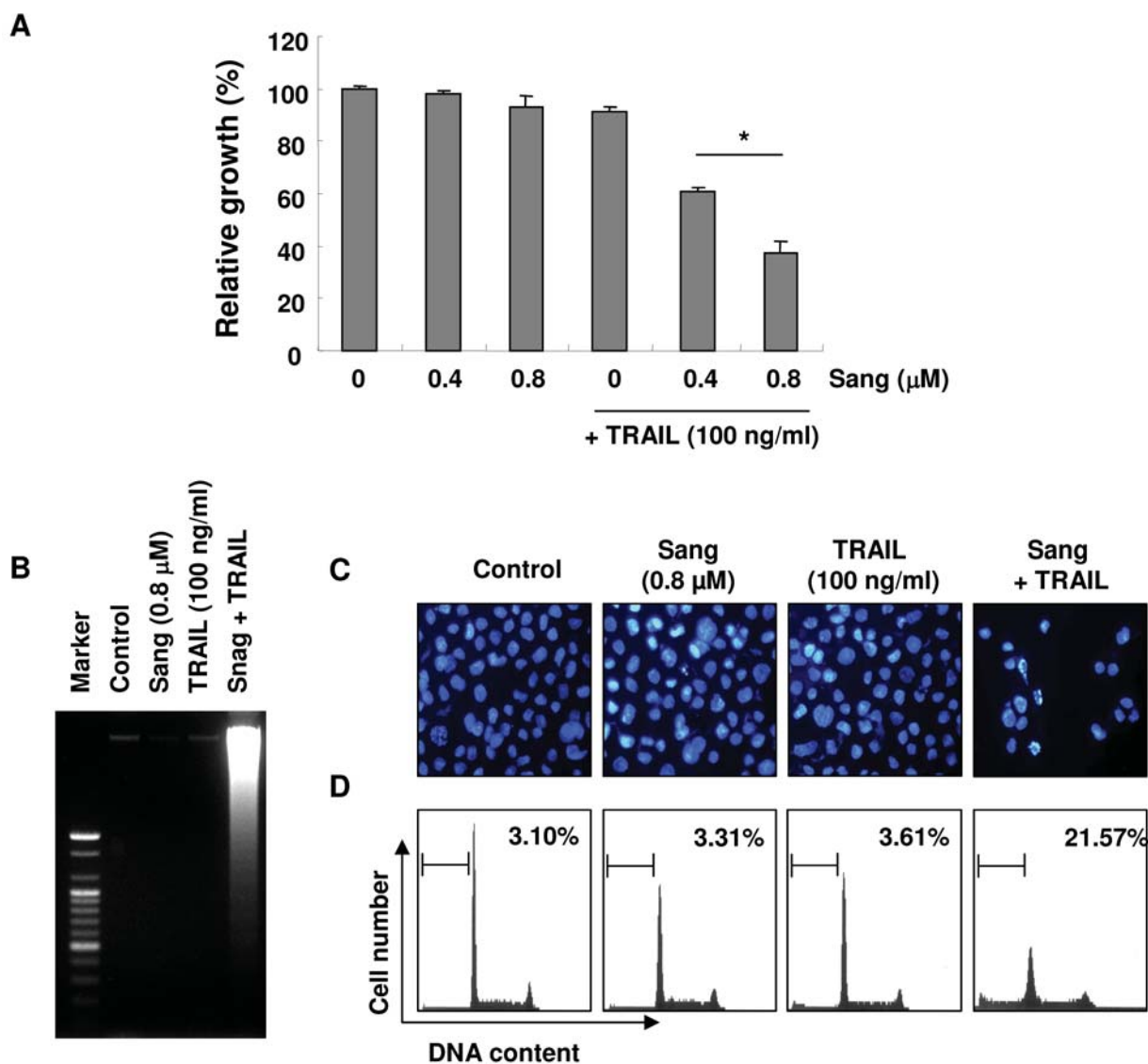


Figure 1. Inhibition of cell viability and induction of apoptosis by combined treatment with sanguinarine and TRAIL in TRAIL-resistant AGS cells. The cells were seeded at 1×10^6 cells/ml and then treated with the indicated concentrations of sanguinarine (Sang), TRAIL, or sanguinarine and TRAIL for 24 h. A, Cell viability was determined by MTT assays. Each point represents the mean \pm SD of three independent experiments. The significance was determined by Student's t-test (* $p < 0.05$ versus untreated control). B, To analyze the DNA fragmentation, the cells were treated with sanguinarine, TRAIL or sanguinarine and TRAIL for 24 h. Fragmented DNA was extracted and analyzed on 1.5% agarose gel containing EtBr. C, The apoptotic bodies were examined under a fluorescence microscope ($\times 400$) by DAPI staining. D, The apoptotic sub- G_1 cell population was analyzed by a flow cytometer.

for cleavage of PARP and subsequent apoptosis (20-22). To further evaluate the significance of caspase activation in combined treatment, we used a general and potent inhibitor of caspase-3, z-DEVD-fmk. As shown in Figure 4A, sanguinarine-mediated enhancement of TRAIL-induced cell death was significantly suppressed by z-DEVD-fmk, indicating that the TRAIL-induced apoptosis by sanguinarine was mediated through caspase-3 activation. We also assessed the effect of the combined treatment on

the cell cycle distribution in the presence of z-DEVD-fmk by flow cytometry. As shown in Figure 4B, the combined treatment resulted in a significant accumulation of cells with sub- G_1 DNA content ($20 \pm 3\%$). However, cells treated with sanguinarine and TRAIL in the presence of z-DEVD-fmk had a normal cell cycle profile and did not exhibit an increase in cells with sub- G_1 DNA content. Consistent with the flow cytometry results, combined treatment with sanguinarine and TRAIL also significantly induced

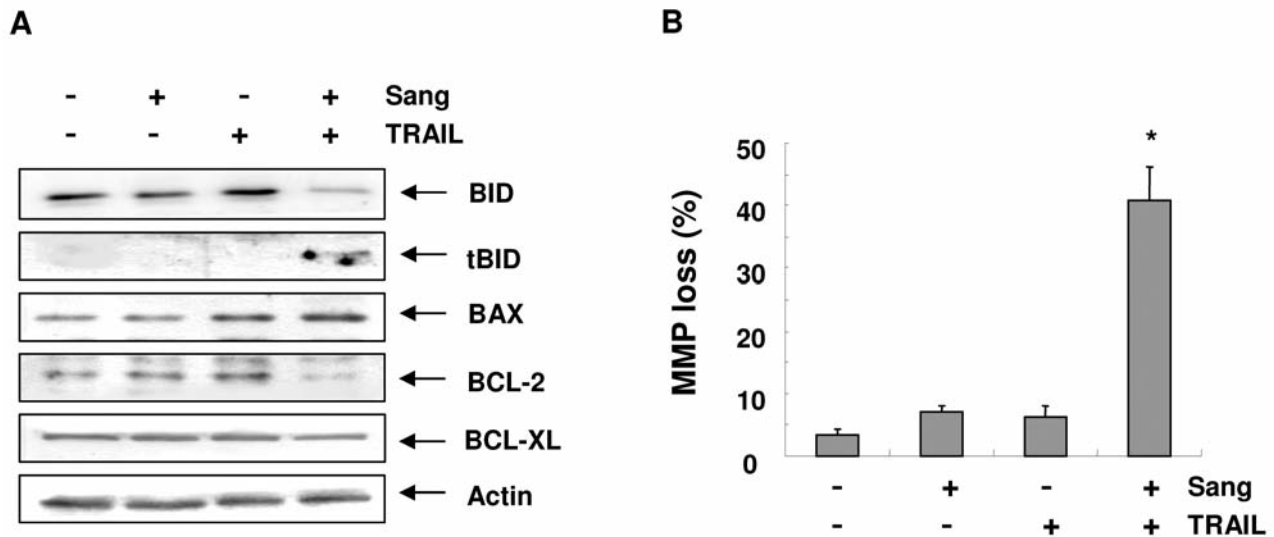


Figure 2. Effects of the combined treatment of sanguinarine and TRAIL on the expression of BCL-2 family proteins and a loss of MMP in AGS cells. AGS cells were treated with the indicated concentrations of sanguinarine and/or TRAIL for 24 h. A, Equal amounts of cell lysate (20 μ g/ μ l) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and probed with anti-BID, anti-BCL-2, anti-BAX, anti-BCL-XL and anti-actin antibodies. Actin was used as the internal control. B, The cells were stained with JC-1 and incubated at 37°C for 20 min. The mean JC-1 fluorescence intensity was detected using a flow cytometer. Data represent the mean \pm SD of representative experiments performed at least three times. The significance was determined by Student's *t*-test (**p*<0.05 versus untreated control).

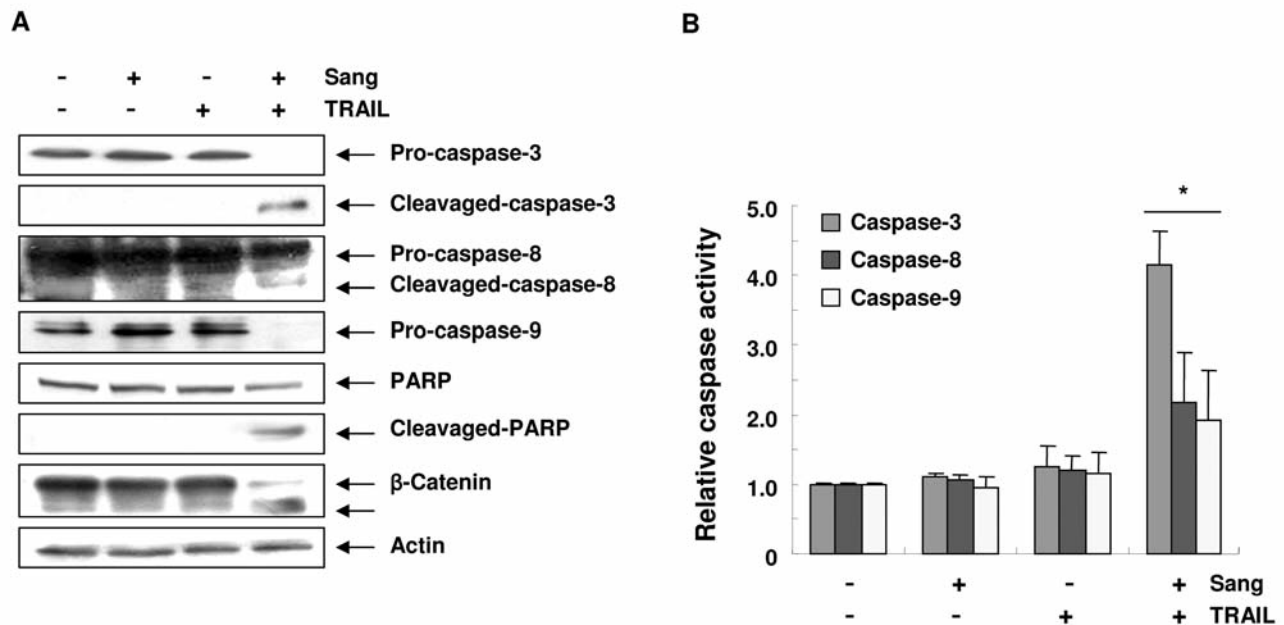


Figure 3. Activation of caspases and alteration of caspase target molecules by the combined treatment of sanguinarine and TRAIL in TRAIL-resistant AGS cells. AGS cells were treated with the indicated concentrations of sanguinarine and/or TRAIL for 24 h. A, Equal amounts of cell lysate (20 μ g/ μ l) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and probed with anti-caspase-3, anti-caspase-8, anti-caspase-9, anti-PARP, anti- β -catenin, and anti-actin antibodies. Actin was used as the internal control. B, Caspase-3, -8, and -9 activities were determined following the manufacturer's protocol. Each point represents the mean \pm SD of three independent experiments. The significance was determined by Student's *t*-test (**p*<0.05 versus untreated control).

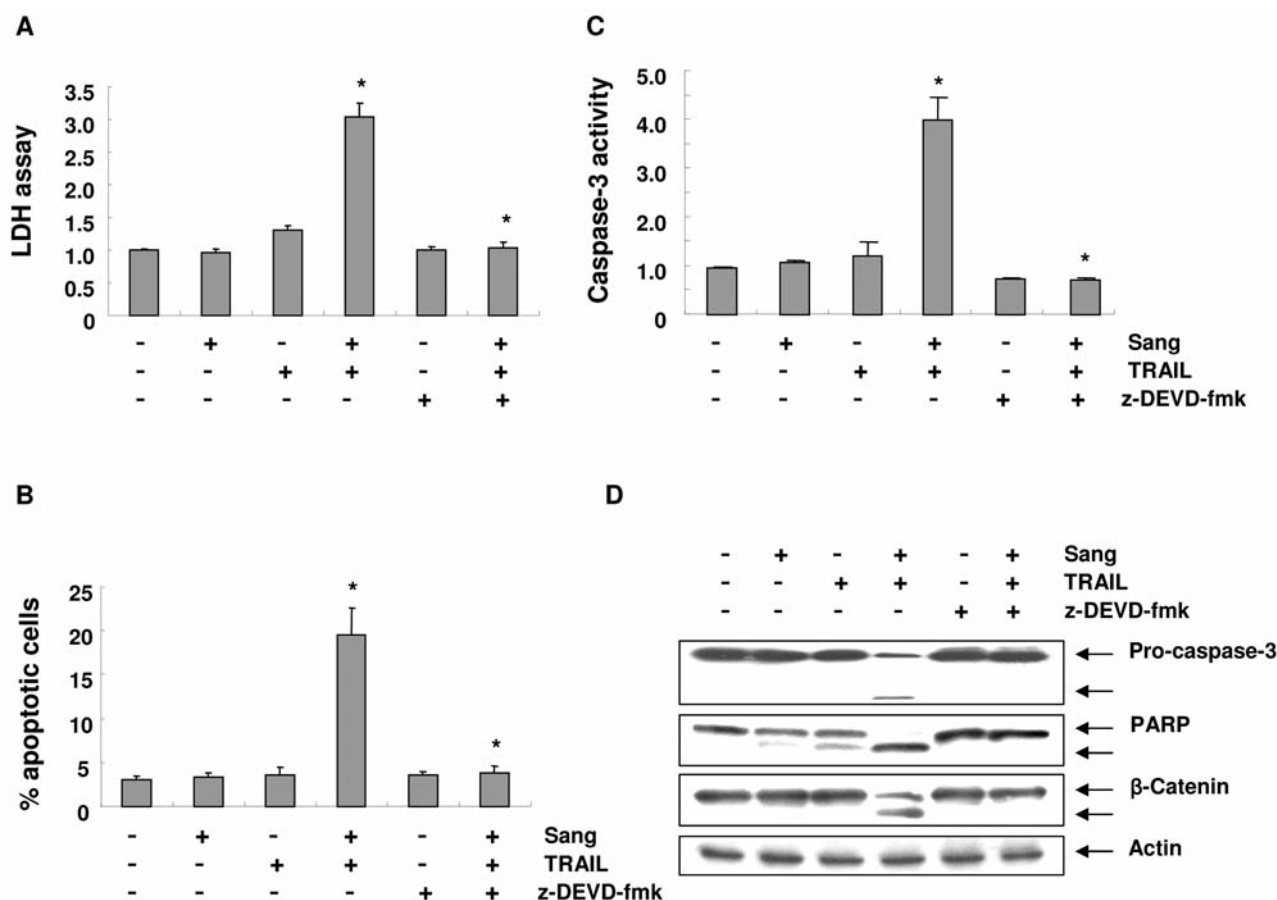


Figure 4. Inhibition of combined treatment-induced apoptosis by the caspase-3 inhibitor, z-DEVD-fmk, in AGS cells. AGS cells were co-incubated with sanguinarine and TRAIL for 24 h after 1 h pre-treatment with z-DEVD-fmk (50 μ M). A, LDH assay was determined following the manufacturer's protocol. B, The sub- G_1 population was analyzed by a flow cytometer. C, Caspase-3 activity was determined following the manufacturer's protocol. Each point represents the mean \pm SD of three independent experiments. The significance was determined by Student's *t*-test (* p < 0.05 versus untreated control). D, Equal amounts of cell lysate (20 μ g/ μ l) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and probed with anti-caspase-3, anti-PARP, anti- β -catenin, and anti-actin antibodies. Actin was used as the internal control. The results are from one representative experiment of three performed.

caspase-3 activity (Figure 4C), and cleavage of PARP, β -catenin and caspase-3 (Figure 4D), whereas z-DEVD-fmk pre-treatment markedly inhibited cleavage of PARP, β -catenin, and caspase-3 and activation of caspase-3. These results clearly indicate that sanguinarine partially sensitizes AGS cells to TRAIL-mediated apoptosis through caspase-3 activation.

Blockage of the AKT pathway increases apoptosis induced by combined treatment with sanguinarine and TRAIL. To investigate the significance of the AKT pathway in response to the combined treatment, we determine the expression and phosphorylation levels of AKT. As shown in Figure 5A, the levels of phosphorylated AKT significantly decreased in response to sanguinarine and

TRAIL at 24 h. Consistent with this result, the expression levels of AKT also decreased but not when sanguinarine and TRAIL were used alone. We next investigated whether the activation of the AKT pathway is necessary for apoptosis induced by the combined treatment. The PI3K inhibitor LY294002 (AKT-upstream inhibitor) was used to determine whether the inhibition of AKT phosphorylation was responsible for the induction of apoptosis. As shown in Figure 5B, pre-treatment with LY294002 in combination with sanguinarine and TRAIL resulted in a marked increase in cell death, as determined by cell viability. Consistent with this result, pre-treatment with LY294002 significantly up-regulated pro-apoptotic BAX expression and cleavage of caspase-3, PARP and β -catenin, while down-regulating phosphorylated AKT as well as total AKT proteins

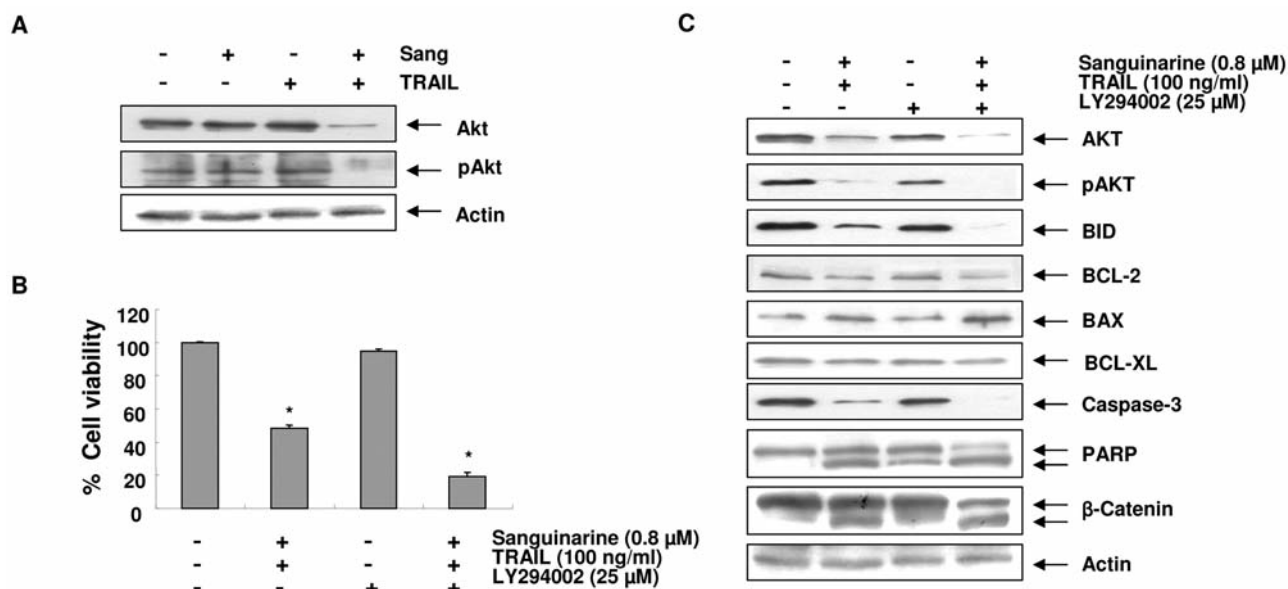


Figure 5. Enhancement of combined treatment-induced apoptosis by the PI3K inhibitor, LY294002, in AGS cells. AGS cells were treated with indicated concentrations of sanguinarine and/or TRAIL for 24 h. LY294002 (25 μM) was given 1 h before the combined treatment. **A**, Equal amounts of cell lysate (20 μg/μl) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and probed with anti-AKT, anti-pAKT, and anti-actin antibodies. Actin was used as an internal control. **B**, Cell viability was determined by MTT assays. Each point represents the mean±SD of three independent experiments. The significance was determined by Student's *t*-test (**p*<0.05 versus untreated control). **C**, Equal amounts of cell lysate (20 μg/μl) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and probed with the indicated antibodies. Actin was used as an internal control.

compared with the combined treatment (Figure 5C). LY294002 alone did not. These results indicate that combined treatment-induced apoptosis may be associated with down-regulation of the AKT signaling pathway.

Discussion

Recently, several groups identified TRAIL as a family that induces apoptosis in many types of cancer cells (1, 2). Activation of caspase-8 by TRAIL leads to two different apoptotic pathways, depending on the cell type. TRAIL induces apoptosis in a mitochondrial-independent manner by activating downstream effector caspases such as caspase-3, whereas a mitochondrial-dependent pathway proceeds *via* activation of caspase-9, which then induces the execution phase of apoptosis (3, 17, 18, 20). Our results indicate that the combined treatment of sanguinarine and TRAIL simultaneously induces mitochondrial-dependent (caspase-9) and independent (caspase-8) apoptosis.

TRAIL may be a safe and effective biological agent for cancer therapy in humans; however, some cancer cells, such as these of the AGS cell line, are known to be resistant to TRAIL-induced apoptosis (13, 14). Several reports have also shown that chemotherapeutic agents sensitized TRAIL-induced synergistic cytotoxicity in TRAIL-resistant cells (14-

16, 23-25). Thus, it is very important to seek an agent that can sensitize TRAIL-induced apoptosis in TRAIL-resistant cancer cells. In the present study, we demonstrated that combined treatment with sanguinarine and TRAIL triggers apoptosis in AGS cells that are normally resistant to either agent alone. Furthermore, this sensitizing effect occurred in a sanguinarine dose-dependent manner. Our findings also indicated that caspases are critical protease mediators of apoptosis triggered by the combined treatment of sanguinarine and TRAIL.

Caspases belong to a family of cysteine proteases that are integral parts of the apoptotic pathway. In particular, activated caspase-3 has many cellular targets that, when severed and/or activated, produce the morphological features of apoptosis. Caspase activation is regulated by various cellular proteins, including BCL-2 family proteins (19-22). Executioner caspases cleave PARP and β-catenin, which are marker proteins for apoptosis (21, 22). Although other groups have reported that TRAIL alters expression of the anti-apoptotic proteins, especially BCL-XL (26-28), we did not observe significant down-regulation of BCL-XL levels in the experiments. However, we did find that the levels of pro-apoptotic BAX protein were increased and those of anti-apoptotic BCL-2 were inhibited in cells treated with sanguinarine and TRAIL together. Combined

treatment with sanguinarine and TRAIL also resulted in truncation of the pro-apoptotic BID protein. In addition, the combination of sanguinarine and TRAIL caused an increase in caspase activity and β -catenin and PARP cleavage.

Most of the signals for survival trigger growth factor receptors, which activate the PI3K/Akt pathway and promote cell growth (29, 30). Our results showed that the combined treatment significantly down-regulated the activation of AKT. These results are in opposition to our other recent reports that indicate treatment with sanguinarine alone resulted in AKT down-regulation and the induction of apoptosis in C6 rat glioblastoma cells (12). It is possible that the disparities in expression patterns are due to the combined treatment rather than to differences in cell type. These results show that the PI3K/Akt signaling pathway is important for cell death induced by the combined treatment with TRAIL in AGS cells.

Resistance to apoptosis is a major obstacle to chemotherapeutic treatment of cancer. The ability to induce apoptosis makes sanguinarine a potentially effective preventative and therapeutic agent to combat malignancy. Although TRAIL represents another potentially important novel anticancer agent, recent studies have shown that many cancer cells are resistant to the apoptotic effects of TRAIL. Thus, combined treatment with sanguinarine and TRAIL may offer a good strategy for the treatment of a variety of human tumors that are resistant to chemotherapy or TRAIL treatment alone. Taken together, the results of this study suggest that sanguinarine sensitizes cells to TRAIL-mediated apoptosis through the up-regulation of apoptotic proteins, including BAX and BID, and activation of caspases, which is mediated by the AKT signaling pathway in AGS cells. In conclusion, the use of TRAIL in combination with subtoxic doses of sanguinarine may provide an effective therapeutic strategy for safely treating some TRAIL-resistant gastric cancer cells.

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