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Research article

Effect of sun ginseng potentiation on epirubicin and paclitaxel-induced apoptosis in human cervical cancer cells

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ABSTRACT

Background: Sun ginseng (SG), a specific formulation of quality-controlled red ginseng, contains approximately equal amounts of three major ginsenosides (RK1, Rg3, and Rg5), which reportedly has antitumor-promoting activities in animal models.
Methods: MTT assay was used to assess whether SG can potentiate the anticancer activity of epirubicin or paclitaxel in human cervical adenocarcinoma HeLa cells, human colon cancer SW111C cells, and SW480 cells; apoptosis status was analyzed by annexin V-FITC and PI and analyzed by flow cytometry; and apoptosis pathway was studied by analysis of caspase-3, -8, and -9 activation, mitochondrial accumulation of Bax and Bak, and cytochrome c release.
Results: SG remarkably enhances cancer cell death induced by epirubicin or paclitaxel in human cervical adenocarcinoma HeLa cells, human colon cancer SW111C cells, and SW480 cells. Results of the mechanism study highlighted the cooperation between SG and epirubicin or paclitaxel in activating caspase-3 and -9 but not caspase-8. Moreover, SG significantly increased the mitochondrial accumulation of both Bax and Bak triggered by epirubicin or paclitaxel as well as the subsequent release of cytochrome *c* in the targeted cells.
Conclusion: SG significantly potentiated the anticancer activities of epirubicin and paclitaxel in a synergistic manner. These effects were associated with the increased mitochondrial accumulation of both Bax and Bak that led to an enhanced cytochrome *c* release, caspase-9/-3 activation, and apoptosis. Treating cancer cells by combining epirubicin and paclitaxel with SG may prove to be a novel strategy for enhancing the efficacy of the two drug types.

1. Introduction

Cancer is one of the most fatal diseases that poses a threat to human health worldwide [1]. A deviant regulation of apoptosis is required for cancer initiation, development, and metastasis [2]. Recent anticancer treatment, including chemotherapy, immunotherapy, radiation, and cytokines, primarily induce apoptosis in targeted cancer cells [3]. Apoptosis, a programmed cell death, is initiated through two main pathways: the exogenous pathway, which is characterized by death receptor activation; and the endogenous pathway, which is characterized by mitochondrial destruction [4]. The tumor necrosis factor receptor superfamily triggers the membrane receptor aggregation and then recruits Fas associated death domain (FADD) and caspase-8 by binding of its specific ligand. Upon recruitment, caspase-8 becomes activated and initiates apoptosis through the direct cleavage of the downstream effector caspases, particularly caspase-3 and -7. In the mitochondrial pathway, apoptogenic factors, such as cytochrome c, second mitochondria-derived activator of caspases (Smac), or apoptosis-inducing factor (AIF), are released into the cytosol from the mitochondria. Cytochrome c triggers the activation of caspase-9 by forming the cytochrome c/apoptotic protease-activating factor

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(Apaf-1)/caspase-9-containing apoptosome complex. Meanwhile, Smac promotes the activation of caspase by invaliding the inhibitory effects of the inhibitors of apoptosis (IAP) family [5–7]. Combination treatments prove to be advantageous in treating malignancies that still partially respond to a single treatment [8]. Drugs have long been combined to treat diseases and reduce suffering; this long-standing history of drug combinations is clearly depicted in traditional Chinese medicines [9].

Panax ginseng has been long used for several thousand years in the Orient as a tonic, prophylactic, and restorative agent [10]. Sun ginseng (SG), a new type of ginseng that is processed by heating at specific pressures, contains approximately equal amounts of three major ginsenosides (RK1, Rg3, and Rg5). SG reportedly serves several functions, including radical scavenging and antitumor-promoting activities [11–13]. Ginsenoside Rg3, one of the most studied of the three major ginsenosides, is reported to inhibit the proliferation, migration, and invasion in many common cancers, such as liver, prostate, colorectal, and ovarian, and has been widely used in cancer treatment [14–17]. Rg3 can induce apoptosis and cell cycle arrest in different cancer cells via different pathways such as downregulating hypoxia inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) [18–21]. Rk1 was investigated to inhibit telomerase activity and cell growth and induce apoptosis through activation of caspase-8 and -3 via ERK pathway, whereas another article demonstrated that Rk1 could induce G1 arrest and autophagy [22,23]. Rg5 blocks the cell cycle at the Gl/S transition phase by increasing p21Cip/WAF1 and decreasing cyclin E and CDK2 [24].

Epirubicin is a third-generation anthracycline that treats a broad spectrum of cancers, including cervical, breast, lung (especially small cell lung cancer), ovarian, stomach, colon, and bladder, and malignant lymphoma [25,26]. Similar to widely used anticancer drugs, epirubicin exhibits some adverse effects on blood, the stomach, and the heart; these effects largely depend on the applied doses [27]. Paclitaxel is another important anticancer drug that is widely used as a chemotherapeutic agent for treating ovarian, breast, lung, colorectal, bladder, prostate, and gastric cancer, melanoma, and lymphoma [28–30]. Paclitaxel, which is an inhibitor of microtubule degradation, induces cell cycle arrest at the G2/M phase [31,32] and ultimately apoptosis [33,34]. This drug also has significant adverse effects, such as hypersensitivity, neutropenia syndrome, neurotoxicity, heart rhythm disorders, and intracellular toxicity [35–37]. Therefore, developing adjuvant agents to potentiate the anticancer activities of epirubicin and paclitaxel and to minimize their adverse effects is significant.

In the current study, SG significantly potentiated the anticancer activities of epirubicin and paclitaxel in a synergistic manner. These effects were associated with the increased mitochondrial accumulation of both Bax and Bak that led to an enhanced cytochrome c release, caspase-9/-3 activation, and apoptosis.

2. Materials and methods

2.1. Materials

SG was provided by Dr. Jeong Hill Park, College of Pharmacy, Seoul National University, Seoul, Korea. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and dimethylsulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Epirubicin was acquired from Pfizer (Wuxi, China). Newborn calf serum and Dulbecco modified Eagle's medium (DMEM) were purchased from Gibco (Life Technologies, Grand Island, NY, USA). Caspase substrates Ac-DEVD-AFC, Ac-IETD-AFC, and Ac-LEHD-AFC were purchased from Calbiochem (La Jolla, CA, USA). The Mitochondria Isolation Kit was purchased from Pierce (Rockford, IL, USA). Annexin V-FITC Apoptosis Detection Kit was purchased from KeyGEN Biotech (Nanjing, China). Polyvinylidene difluoride (PVDF) membrane was purchased from Millipore (Billerica, MA, USA). Antibodies against cytochrome c, poly (adenosine diphosphate-ribose) polymerase (PARP), Bak, Bax, α-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against caspase-8, -9, and cytochrome c oxidase II (Cox II) were purchased from Cell Signaling Technology (Beverly, MA, USA). Clarity Western ECL Substrate Kit was purchased from Bio-Rad (Hercules, CA, USA).

2.2. Cell culture

HeLa, SW111C, and SW480 cells were grown in DMEM supplemented with 10% (by volume) heat-inactivated newborn calf serum, 100 μg/mL of streptomycin 100 U/mL of penicillin, at 37°C in a humidified atmosphere with 5% CO_2.

2.3. Analysis of SG methanol extract

The SG methanol extract was analyzed as a previous report described [38]. Briefly, SG was dissolved in MeOH (3 mg/mL), and filtered with 0.45μm Millipore filter, and the solution was analyzed with a Waters 2695 liquid chromatograph (Waters Corporation, Milford, MA, USA) fitted with Knauer C-18, reverse-phase column (Knauer, Berlin, Germany; 5μm,φ250 mm × 3 mm) utilizing the solvent gradient system. The mobile phase consisted of acetonitrile water (Solvent A) and water (Solvent B) and the flow rate was 0.6 mL/min. The detector was a Waters 2996 PDA Detector (Waters Corporation). The gradient elution was used as follows: 0–20 min, 20% A; 20–31 min, linear gradient from 20–32% A; 31–40 min, linear gradient from 32–43% A; 40–70 min, linear gradient from 43–100% A; and 70 min, 100% A.

2.4. MTT assay

Exponentially growing cells were seeded into a 96-well plate at 0.8×10^4 cells/well in triplicate. After incubation for 20 h, cells were treated with increasing concentrations of SG, epirubicin, or paclitaxel for 48 h. At 44 h posttreatment, 20 μL of MTT (5 mg/mL) was added to each well and incubated for 4 h. Then 150 μL of DMSO was added to every well to solubilize the formazan crystals formed by viable cells, and the color intensity was measured at 550 nm with an enzyme-linked immunosorbent assay plate reader (TECAN, Männedorf, Switzerland).

2.5. Apoptosis assay

HeLa cells were cultured for 20 h and then treated with 80 μg/mL SG with 0.5 μg/mL epirubicin or 10nM paclitaxel alone or combined for 24 h. HeLa cells were harvested, washed with ice-cold phosphate buffered saline (PBS), and stained with annexin V/PI reagent as described previously [3]. The percentage of annexin V (+) cells was determined by flow cytometry (Becton Dickinson FACS Calibur Cytometer, San Jose, CA, USA). The percentage of annexin V (+) cells indicates the frequency of total apoptotic cells.

2.6. Cell-free caspase activity assay

As described [39], HeLa were treated and harvested. 50 μg whole-cell lysates were incubated with 200nM Ac-LEHD-AFC (for caspase-9), Ac-IETD-AFC (for caspase-8), and Ac-DEVD-AFC (for caspase-3) in a reaction buffer containing 20mM