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Dossier : Anticancer compounds and drug resistance circumvention

Mitochondria-mediated apoptosis in human breast carcinoma MCF-7 cells induced by a novel selenadiazole derivative

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Abstract

The role of organoselenium compounds as potent cancer chemopreventive and chemotherapeutic agents has been supported by epidemiological, preclinical and clinical studies. In this study, a novel selenadiazole derivative, 1,2,5-selenadiazolo-[3,4-d]pyrimidine-5,7-(4H,6H)-dione (SPO), is identified as a potent antiproliferative agent against human breast adrenocarcinoma MCF-7 cells, human hepatoma HepG2 cells and human melanoma A375 cells. Induction of apoptosis in MCF-7 and A375 cells by SPO was evidenced by accumulation of sub-G1 cell population, DNA fragmentation and nuclear condensation. Further investigation on intracellular mechanisms found that SPO treatments induced activation of caspase-8 and caspase-9, overproduction of reactive oxygen species, and depletion of mitochondrial membrane potential ($\Delta\Psi m$) through regulating the expression of pro-survival and pro-apoptotic Bcl-2 family members. Our findings suggest that SPO is a promising novel organoselenium compound with potential in the treatment of human cancers.

Keywords: Selenium; Selenadiazole derivative; Apoptosis; Mitochondrial dysfunction; Reactive oxygen species

1. Introduction

The trace element selenium (Se) is an essential nutrient of fundamental importance to human and animal. Epidemiological, preclinical and clinical studies support the role of Se compounds as potent cancer chemopreventive agents [1]. Se supplementation was found effective in reducing the incidence of cancers including prostate, lung, colon and liver cancers [2,3]. Many studies have showed that the dose and the chemical form of Se were the most critical for cancer chemoprevention [4,5]. Several mechanisms have been postulated to elucidate the anticancer activity of Se [6], which included induction of cell apoptosis and inhibition on cell proliferation, maintenance of glutathione peroxidase activity and modulation of redox state, prevention and detoxification of carcinogenic intermediate metabolites, stimulation of the immune system and inhibition of angiogenesis. Among them, cell apoptosis has been postulated as one of the most critical mechanism for chemopreventive action of Se [1].

The application of synthetic organoselenium compounds in chemoprevention and chemotherapy is a fascinating field for cancer research [7]. During the past decade, a lot of potent organoselenium compounds have been designed to achieve greater chemopreventive efficacy and minimal side effects by structural modifications, such as ebselen, selenocyanate, selenobetaine and Se analogues of amino acids and other sulfur compounds with known antitumor activity [1,7,8]. Se-containing heterocyclic compounds have attracted more and more attention for their pharmacological potential and interesting chemical properties [9]. For instance, a number of selenazolo derivatives, for example, ebselen, 4-Methyl-1,2,3-selenadiazole-5-carboxylic acid amides and 6-phenyl-7 (6H)-isoselenazolo [4,3-d] pyrimidone, have been synthesized and found to

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show *in vitro* and *in vivo* antitumor activities [10–14]. However, so far very little information about the cell apoptosis and the underlying mechanisms triggered by selenazolo derivatives could be obtained.

In our previous works, above 20 selenadiazole derivatives were designed and synthesized from corresponding ortho-aromatic diamines by using optimized solid state synthesis methods [15–17]. In the preliminary screening, 1,2,5-selenadiazolo-[3,4-d]pyrimidine-5,7-(4H,6H)-dione (SPO) was found to be selectively cytotoxic toward cancer cells. Thus, the objectives of this study are to examine the antiproliferative and apoptosis inducing activities of SPO and to elucidate the molecular mechanisms for the apoptotic cell death induced by SPO in selected cancer cells.

2. Materials and methods

2.1. Chemicals and antibodies

Thiazolyl blue tetrazolium bromide (MTT), propidium iodide (PI), solid JC-1, 4′,6-Diamidino-2-phenyindole (DAPI), 2′,7′-dichlorofluorescein diacetate (DCF-DA), bicinchoninic acid kit for protein determination and selenomethionine were purchased from Sigma. Antibodies of Bax and Bcl-2 were purchased from Cell Signaling Technology (Beverly, MA). Caspase-9 substrate (Ac-LEHD-AFC) and caspase-8 substrate (IETD-AFC) were purchased from Calbiochem.

2.2. Synthesis of SPO

1,2,5-selenadiazolo-[3,4-d]pyrimidine-5,7-(4H,6H)-dione (SPO) was synthesized from corresponding ortho-aromatic diamines by using an optimized microwave-assisted solid state synthesis method [15–17]. The obtained compound was shown by GC-MS analysis to be at least 98% pure [18]. A 20 mM stock solution of SPO was prepared in dimethyl sulfoxide (DMSO) and stored at −20 °C. The final concentration of DMSO in SPO-containing medium was less than 0.5%, which caused no cytotoxic effects on all cells tested in this study.

2.3. Cell culture

The cell lines used in this study, including human breast adrenocarcinoma MCF-7 cells, human liver cancer Hep G2 cells, human melanoma A375 cells and the normal cells Hs68 human fibroblasts were obtained from American Type Culture Collection (Manassas, VA). Cells were maintained in either RPMI 1640 or DMEM media supplemented with 10% fetal bovine serum, penicillin (100 units/ml) and streptomycin (50 units/ml) at 37 °C in a humidified incubator with 5% CO_2 atmosphere.

2.4. Cell viability examination

The effect of SPO on cell proliferation was determined by MTT assay. Briefly, cells were seeded in 96-well culture plates at different densities. After 24 h, different concentrations of SPO were added and incubated for indicated time. Then 20 μl/well of MTT solution (5 mg/ml phosphate buffered saline) was added and incubated for 5 h. The medium was removed and replaced with 150 μl/well of acidic isopropanol (0.04 M HCl in isopropanol) to dissolve the formazan crystals. Absorbance at 570 nm was taken on a 96-well microplate reader.

2.5. Cytotoxicity detection by lactate dehydrogenase (LDH) assay

Release of the cytoplasmic enzyme LDH from the damaged cells into the culture media was used as a marker for cell membrane integrity and could be used to detect the cytotoxicity [19]. In this assay, cells treated or untreated were precipitated by centrifugation at 1500 × *g* for 10 min at room temperature. Then 100 μl of the supernatants was transferred into new wells, and LDH was determined by using the Cytotoxicity Detection kit (Roche, Germany) according to the manufacturer's guidelines. Cytotoxicity was evaluated by measuring the percentage of LDH released into the medium. Cells treated with 1% Triton X-100 were used as high control (100% lysis).

2.6. Flow cytometric analysis

Cell cycle distribution was monitored by flow cytometric analysis. Briefly, cells cultured with or without SPO, were harvested and washed with PBS. Cells were stained with propidium iodide (PI) after fixed with 70% ethanol at −20 °C overnight. Data acquisition was performed by a Beckman Coulter Epics XL MCL flow cytometer (Miami, FL). The cell cycle distribution was analyzed using MultiCycle software (Phoenix Flow Systems, San Diego, CA). The proportion of cells in G0/G1, S, G2/M phases was represented as DNA histogram. Apoptotic cells with hypodiploid DNA content were measured by quantifying the sub-G1 peak in cell cycle pattern. For each experiment, 10,000 events per sample were recorded.

2.7. TUNEL assay and DAPI staining

DNA fragmentation was examined with fluorescence staining by the TUNEL apoptosis detection kit (Roche) following the manufacturer's instruction. Briefly, cells cultured in chamber slides were fixed with 3.7% formaldehyde and permeabilized with 0.1% Triton X-100 in PBS. The cells were incubated with TUNEL reaction mixture for 1 h. For nuclear staining, cells were incubated with 1 μg/ml of DAPI for 15 min at 37 °C. The cells were then washed with PBS and examined on a fluorescence microscope (Nikon Eclipse 80i).

2.8. Evaluation of mitochondrial membrane potential ($\Delta\Psi m$)

Cells cultured in 6-well plates were trypsinized and resuspended in 0.5 mL of PBS buffer containing 10 μg/ml of JC-1. After incubation for 10 min at 37 °C in the incubator,