European Review for Medical and Pharmacological Sciences 2013; 17: 2166-2171

Sodium selenite induces apoptosis in colon cancer cells via Bax-dependent mitochondrial pathway

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Abstract. – OBJECTIVES: We aimed to elucidate a possible mechanism of action by investigating the effects of selenium (Se) on cell cycle arrest and apoptosis in colorectal cancer cells (HCT116 cells and SW620 cells).

MATERIALS AND METHODS: The colorectal cancer cells were treated with varying concentrations of Se (1 microM, 5 microM and 10 microM) for 24 hours. The effects of Se on cell cycle, apoptosis, mitochondrial transmembrane potential and apoptosis related proteins were examined by flow cytometry assessment and immunoblotting.

RESULTS: Se induced G2/M cell cycle arrest and apoptosis in colorectal cancer cells (HCT116 cells and SW620 cells) in a dose-dependent manner. Bax (Bcl2 associated X protein) was up-regulated and Bcl-2 (B cell linphoma gene-2) was down-regulated after Se treatment in both cells in a dose-dependent manner. Se caused increased loss of MMP (matrix metalloproteinase) and induced Bax translocation from cytosol into mitochondria and caspase 3 activation in both colorectal cancer cells in a dose-dependent manner.

CONCLUSIONS: Se induced G2/M cell cycle arrest and apoptosis in both colorectal cancer cells via Bax-dependent mitochondrial pathway.

Key Words:
Colorectal cancer cells, Sodium selenite, Cell cycle arrest, Apoptosis.

Introduction

Colorectal cancer is a common malignant tumor. In China, it runs after gastric cancer, lung cancer and esophageal cancer. It will become one of the most common malignant tumors with high incidence, increasing morbidity and mortality. Therefore, an important and urgent task for cancer researchers is to further study the pathogenesis of colorectal cancer and find effective chemotherapeutic drugs.

Selenium (Se) is a necessary trace element in mammalian and human. It is well supported that selenium has chemopreventive effects, and emerging evidences suggest that selenium has chemotherapeutic potential by inducing cancer cell apoptosis with minimal side effects to normal cells within a proper dose range[1-5]. However, the precise mechanisms by which selenium activates the apoptotic machinery remain poorly understood[6,7].

Apoptosis is one of the most fundamental processes to life. Essential to several processes ranging from normal development to regulation of the immune system and tissue homeostasis, apoptosis is conserved across all metazoans[8-10]. Deregulated apoptosis has been implicated in a variety of pathological conditions including cancer, neurodegenerative disorders and autoimmune diseases[11]. It has been extensively studied that in mammalian cells the mitochondrial pathway of apoptosis plays a key role in various biological processes.

We demonstrated that Se induces G2/M cell cycle arrest and apoptosis in both colorectal cancer cells via Bax-dependent mitochondrial pathway. We hoped that this would provide new insights for the use of Se in the clinical treatment of colorectal cancers.

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Materials and Methods

Cell Culture and Treatments

HCT116 (human colorectal carcinoma) and SW620 cells were cultured in DMEM medium (Dulbecco's modified eagle's medium) supplemented with 10% fetal bovine serum, 10 mg/ml antibiotics (penicillin and streptomycin) and 2 mmol/L L-glutamine and incubated at 37°C in an atmosphere of 5% CO_2. Sodium selenite (Sigma-Aldrich, St Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO, Sigma), final concentrations of 1 μM, 5 μM and 10 μM were used to treat the cells and proper amount of DMSO was used as vehicle control.

Cell Cycle Analysis

Cells were treated with different concentrations of Se for 24h. After that, cells were digested with 0.25% trypsin and re-suspended in phosfate buffered saline (PBS) at 4°C overnight. Then, cells were washed twice with PBS and treated with 20 μg/ml RNase A at 37°C for 30 min. Finally, cells were stained with 500 μL PI (phenanthridinium intercalator) dye solution at 4°C incubating for 3h and DNA content was analyzed by flow cytometry.

Determination of Mitochondrial Transmembrane Potential

MitoTracker Red probe was used to assess the changes of matrix metalloproteinase (MMP). Under normal circumstances MitoTracker Red accumulated in mitochondria. Once MMP declined, the probe would be released. The probe was dissolved in DMSO, with PBS diluted before use. Cells were treated with MitoTracker for 45 min before trypsinization. Then cells were washed twice with PBS and analyzed by flow cytometry.

Western Blotting

Total protein was extracted using a radioimmunoprecipitation assay (RIPA) agent according to the instructions and was quantified with a bicinchorinic acid (BCA) protein quantity kit. Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 4°C and transferred onto nitrocellulose membranes. After nonspecific sites were blocked with 5% milk-TBST (Tris-Buffered Saline and Tween 20), membranes were incubated with anti-Bcl-2 antibody, anti-Bax antibody, anti-CyclinB1 antibody, anti-CyclinD antibody, anti-active caspase 3 antibody, anti-β-actin or anti-COX4 (cytochrome c oxidase subunit IV). Then membranes were washed in TBST, a horseradish peroxidase-linked antibodywas was employed as a secondary antibody, and the bands of interest were detected using an enhanced chemiluminescence technique. Densities of bands were analyzed by a computer equipped with image software (GEL DOC gel 2000).

Statistical Analysis

Values were calculated as ± SD. A one-way ANOVA was used to analyze differences in all parameters. Significance was considered at $p < 0.05$.

Results

Se Induces G2/M Arrest and Apoptosis in Colon Cancer Cells

We examined the effects of Se treatment on cell cycle distribution in both HCT116 cells and SW620 cells. As shown in Figure 1 A and 1B, the data showed that Se induced dose-dependent increase in G2 phase of treated cells, with decrease of S phase cells, indicating the inhibition of DNA replication. In addition, Western blotting measurement of several important cell cycle markers showed that in both cell lines with Se cyclin D1 was downregulated while cyclin B1 was upregulated in a dose-dependent manner (Figure 2 A and B), indicating that Se induced G2/M arrest in HCT116 and SW620 cells.

We tested Se-induced apoptosis in both colon cancer cell lines. As shown in Figure 3, Se caused dose-dependent apoptosis in both HCT116 cells and SW620 cells.

Se Induces Changes of Apoptosis Related Proteins

Bcl-2 family proteins play a central role in the regulation of apoptosis. This family includes antiapoptotic proteins such as Bcl-2 and "multidomain"proapoptotic proteins such as Bax[12,13]. By western blotting, we obtained the fact that Bax was up-regulated after Se treatment in both HCT116 and SW620 cells in a dose-dependent manner (Figure 4A). On the contrary, the antiapoptotic proteins Bcl-2 were down-regulated in both cell lines by Se (Figure 4 B).

Se Induces Apoptosis in Colon Cancer Cells Via Mitochondrial Pathway

There are many cell apoptosis pathways. The mitochondrial pathway is one of classic apopto-

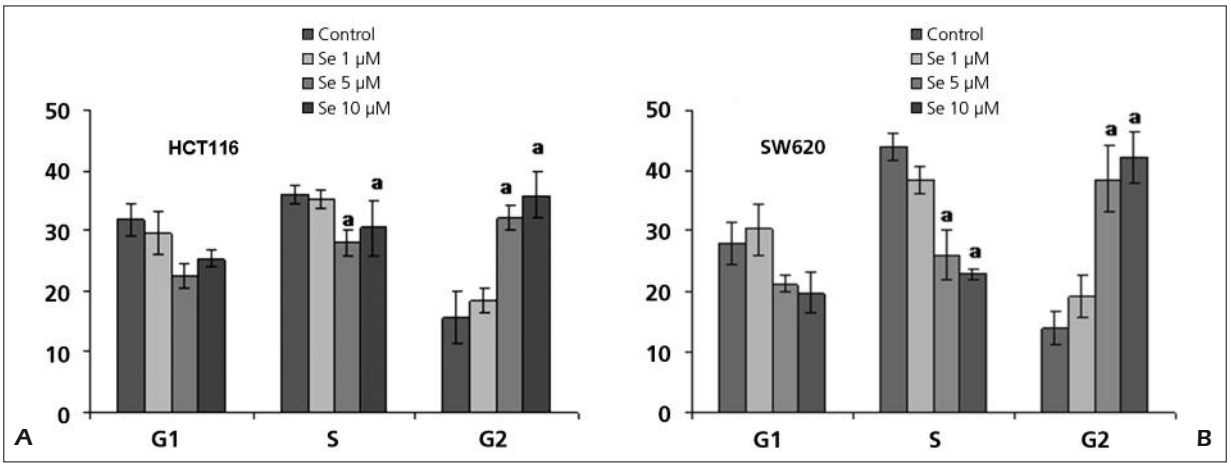

Figure 1. Se induces G2/M arrest in CRC HCT116 and SW620 cells in a dose-dependent way. ***A,*** HCT116 cells. $^{a}p < 0.01$ versus corresponding control. ***B,*** SW620 cells. $^{a}p < 0.01$ versus corresponding control.

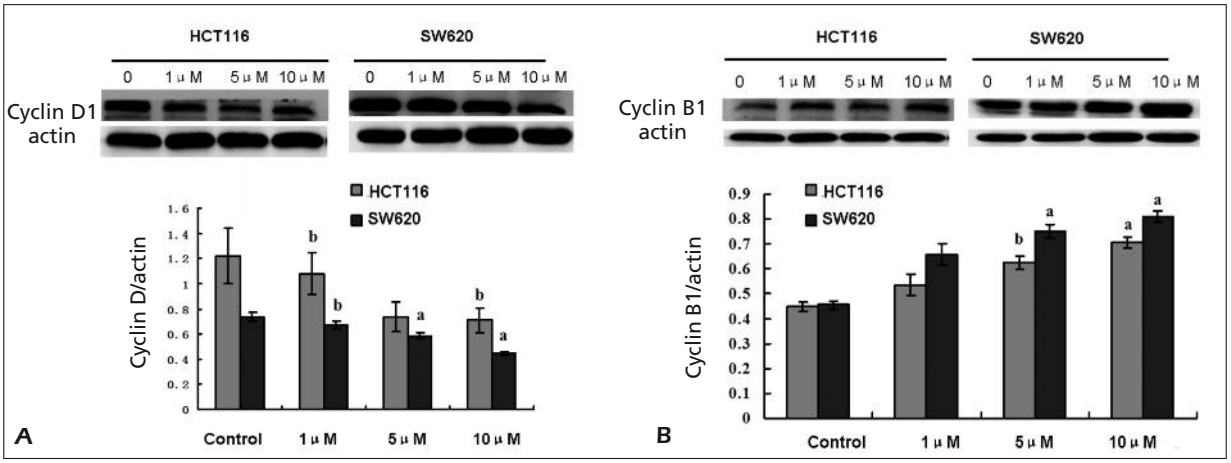

Figure 2. Changes in the expression of cyclin D1 and cyclin B1 in HCT116 and SW620 cells exposed to sodium selenite during 24 h. $^{a}p < 0.01$ versus control ,$^{b}p < 0.05$ versus control.

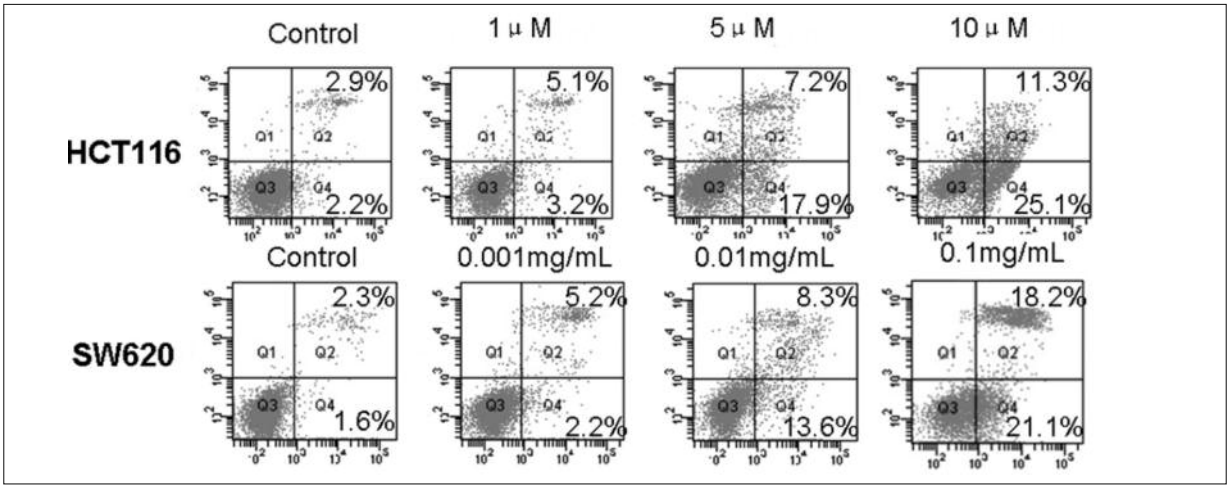

Figure 3. Se induces apoptosis in CRC cell lines. Apoptosis was analyzed by cytometry.

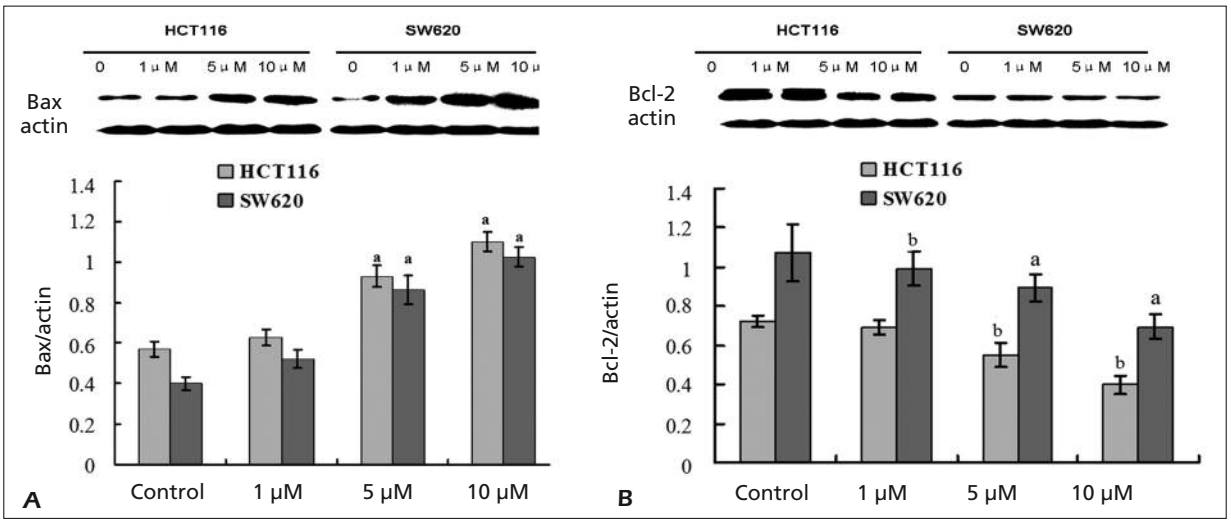

Figure 4. Se induces changes of apoptosis related proteins. Protein from each group of cells was extracted and analyzed by western blotting using antibodies against Bax and Bcl-2. [a]$p < 0.01$ versus control, [b]$p < 0.05$ versus control.

sis pathways. One of the significant features of this pathway is permeabilization of the outer mitochondrial membrane, a process regulated by the Bcl-2 family of proteins[14] and, thus, release of cytochrome c into the cytoplasm to trigger subsequent activation of executioner caspases. Therefore, we doubted whether Se induced apoptosis in colon cancer cells via mitochondrial pathways. Firstly, MMP dissipation was measured. The results showed that Se caused increased loss of MMP in a does-dependent way in both cell lines (Figure 5). Then, Bax levels were measured. The results showed that Se induced both Bax translocation from cytosol into mitochondria in both cell lines (Figure 6 A and B) and there were not much difference between the two cell lines. After that, caspase 3 activation was detected by Western blotting. The results showed that Se induced caspase 3 activation (Figure 7). These findings demonstrated that Se induced apoptosis in both colon cancer cells via mitochondrial pathway, dependent on Bax translocation and caspase 3 activation.

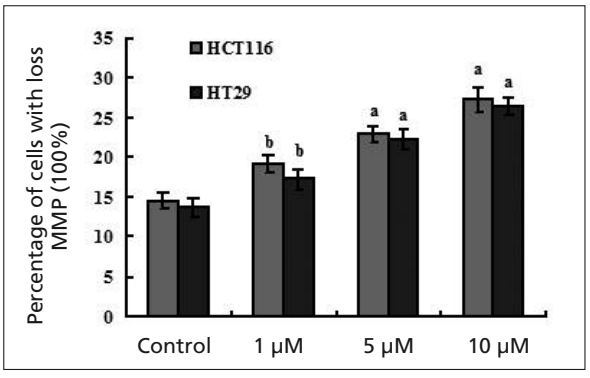

Figure 5. MMP changes was measured by flow cytometry. [a]$p < 0.01$ versus control, [b]$p < 0.05$ versus control.

Discussion

Selenium is widely regarded as a protective agent against cancer risks. Supranutritional levels of selenium have benefits in preventing several types of cancer, including lung cancer, colorectal cancer, and prostate cancer. It is known that conventional chemotherapeutic agents induce apoptosis of cancer cells mostly through p53-dependent pathway. However, p53 mutations and inactivation have been found in more than half of all human carcinoma cells[15]. Therefore, we observed that Se effected on cell cycle in CRC cell lines with wild p53 (HCT116 cells) and mutant p53 (SW620 cells). The results demonstrated that Se induced G2/M phase arrest through dose-dependence in both cells. After examining the changes in Bcl-2 family proteins we found that Se induced the expression of pro-apoptotic protein Bax and decrease the expression of anti-apoptotic protein Bcl-2 in both cell lines. These data indicated that Se induces apoptosis through dose-dependence in colorectal cancer cells.

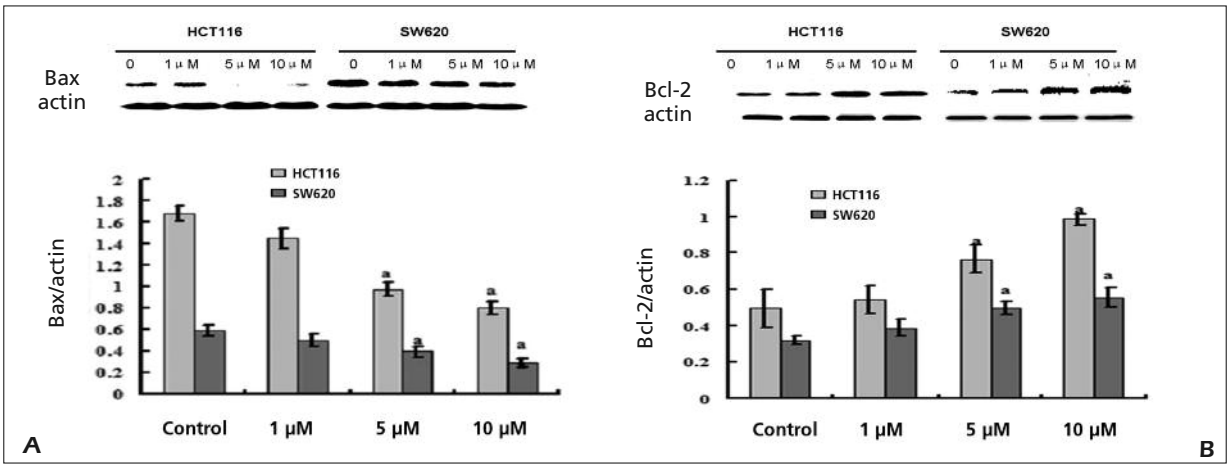

Figure 6. Se induces apoptosis in CRC cells in a Bax-dependent pathway. Changes in the expression of Bax in cytosol and mitochondria. [a]$p < 0.01$ versus control, [b]$p < 0.05$ versus control.

Bax is an initiator in mitochondrial apoptotic pathway. Direct activation of Bax mediates mitochondrial membrane permeabilization, releasing cytochrome c into the cytoplasm to mitochondria, which triggers subsequent activation of executioner caspases, leading to apoptosis[7,16]. Therefore, we doubted whether Se promoted Bax translocation from the cytosol onto mitochondria. The results showed that Se induced Bax translocation from cytosol into mitochondria in both cell lines, suggesting the involvement of mitochondrial pathway. Active caspase 3, the key executor of apoptosis, leads to a series of morphological changes and ultimate apoptotic cell death[7,17]. So the activation of caspase 3 was detected after Se treatment in both cell lines. The results showed that Se induced caspase 3 activation in both cell lines. These results demonstrated that Se induces apoptosis in both CRC cell lines via mitochondrial pathway, dependent on caspase 3 activation regarless of P53.

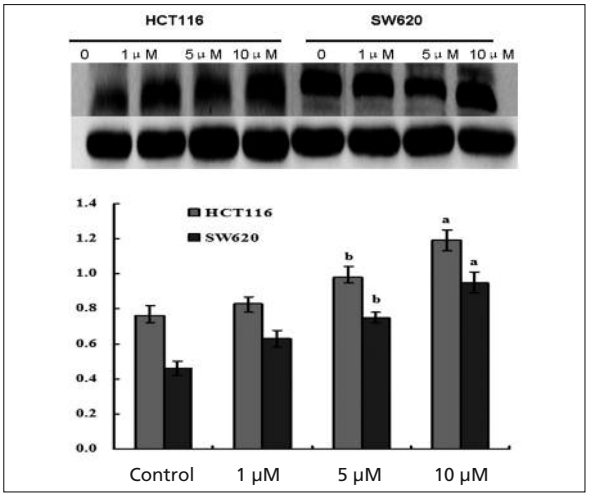

Figure 7. Changes in the expression of caspase-3 in HCT116 and SW620 cells. Protein from each group of cells was extracted and analyzed by western blotting using anti-active caspase-3 antibody. [a]$p < 0.01$ versus control, [b]$p < 0.05$ versus control.

Conclusions

In summary, based on these findings, we conclude that Se induces G2/M phase cell cycle arrest and the mitochondrial pathway of apoptosis in colorectal cancer cells. This study provides useful information for the use of Se to treat clinical patients with colorectal cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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