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Original Research

Antitumor activity of selenium compounds and its underlying mechanism in human oral squamous cell carcinoma cells: A preliminary study

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ABSTRACT

Objective: Oral cancer is an aggressive disease that infiltrates the adjacent tissues and frequently metastasizes to lymph nodes in the neck. Currently, no chemotherapy effectively prevents its metastasis. Selenium compounds have been recently scrutinized as chemotherapeutic agents for various cancers. In this study, we aimed to investigate the antitumor activity of selenium compounds and elucidate the underlying inhibitory mechanism of these agents in oral cancer cells.
Methods: The growth inhibitory effects of selenium compounds (sodium selenite, selenomethionine, and Se-methylselenocysteine) on human oral squamous cell carcinoma (HOSCC) cell lines (HSC-3, HSC-4, and SAS) were evaluated by MTT assay. Selenite-induced apoptosis, caspase activity, and endoplasmic reticulum (ER) stress in HSC-3 cells were evaluated by flow cytometry and western blot. Effects of selenite on Akt expression in HSC-3 cells were evaluated by ELISA and western blot.
Results: Selenium compounds significantly inhibited cell growth and induced apoptosis in HOSCC cell lines. HSC-3 cells, in particular, were highly sensitive to selenite. In selenite-treated HSC-3 cells, caspase-3, 8, and 9 were conspicuously activated; pretreatment with pan-caspase inhibitor or caspase-12 inhibitor dramatically reduced selenite-induced apoptosis; ER stress markers, caspase-12 and eIF-2α, were highly activated, but Akt activation in the Akt/phosphoinositide-3-kinase pathway was downregulated.
Conclusion: Our findings indicate that selenite induces apoptosis in HOSCC cell lines in a caspase-dependent manner through mitochondrial, death-receptor, and ER stress pathways. In addition, selenite could exhibit antitumor activity by downregulating Akt activation, which plays an important role in cell growth and chemotherapy resistance.
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1. Introduction

Selenium compounds are present in soil, and it is well known that the incidence of cancer is lower in areas with selenium-rich soil [1]. Supplementation with selenium compounds during cancer therapy, e.g., radiation or chemotherapy, has been reported to enhance the recovery of cell-mediated immune responsiveness, pointing to a cancer preventive effect of selenium compounds [2]. As selenium compounds were recently shown to possess antitumor effects such as induction of apoptosis and inhibition of cell growth, they may have the potency of chemotherapeutic agents [3]. However, no report has demonstrated the antitumor effects of selenium compounds in oral cancers, especially their effects on the PI3K/Akt pathway, which plays an essential role in cell growth regulation and survival. After growth factors, such as the epidermal growth factor, bind to their receptors, the PI3K/Akt pathway

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is activated, prompting cancer cells to grow. It is widely accepted that Akt plays a crucial role in protecting cells from apoptotic signals and in anticancer drug resistance [4,5], suggesting that Akt's role in antitumor activity should be investigated. Here, we report the effects of selenium compounds on tumor cell growth, apoptosis induction, and Akt activity in human oral squamous cell carcinoma (HOSCC) cells in vitro, and describe the possible mechanism of these effects.

2. Materials and methods

2.1. Cell and culture conditions

HOSCC cell lines including HSC-3, HSC-4, and SAS were obtained from the Cell Resource Center for Biomedical Research (Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan). The cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C in RPMI1640 with 10% fetal calf serum and 0.2% sodium hydrogen carbonate.

2.2. Reagents

Cisplatin (CDDP) (Nippon Kayaku Co., Ltd., Tokyo, Japan) and 5-FU (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) were used as anticancer agents. Sodium selenite (selenite), selenomethionine (SeMet), and Se-methylselenocysteine (MSC) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

The phosphoinositide 3-kinase (PI3K) inhibitor LY294002 was purchased from CST Japan, K.K. (Tokyo, Japan). Pan-caspase inhibitor (z-VAD-fmk) and caspase-12 inhibitor (z-ATAD-fmk) were purchased from the MBL Co., Ltd. (Tokyo, Japan).

2.3. Effects of selenium compounds on OSCC cell growth

The growth inhibitory effects of selenium compounds on OSCC cell lines were evaluated by the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay (MTT colorimetric assay kit, Dojindo Molecular Technologies, Inc., Kumamoto, Japan). HSC-3, HSC-4, and SAS cells were plated in 96-well tissue culture plates (Japan BD Bioscience Inc., Tokyo, Japan) at a density of 2×10^4 cells per well and cultured for 24 h prior to incubation with selenite (1 μM, 10 μM, and 100 μM), MSC (10 μM, 100 μM, and 1000 μM), and SeMet (10 μM, 100 μM, and 1000 μM), respectively. Forty-eight hours later, selenium-treated cells were stained by MTT. The growth inhibitory effects were estimated by measuring the optical density at 450 nm using a microplate reader.

2.4. Selenite-induced apoptosis in OSCC cells

Apoptosis induction was determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of DNA fragments in HSC-3, HSC-4, and SAS cells treated with 5 μM selenite for 2 or 5 days. The TUNEL staining was performed using a Mebstain apoptosis kit (MBL International, Woburn, MA, USA). After treatment, cells were centrifuged and collected, rinsed twice with washing buffer (phosphate-buffered saline containing 0.2% fetal calf serum), fixed with 4% paraformaldehyde in buffer at 4 °C for 30 min, incubated in reaction buffer containing FITC-dUTP and TdT at 37 °C for 60 min, rinsed with washing buffer again, and counted to determine the number of FITC-positive cells (apoptotic cells), using a FACSCalibur flow cytometer (Japan BD Bioscience Inc.).

2.5. Effects of selenite on caspase activity in OSCC cells

HSC-3 cells were treated with 5 μM selenite for 24 h, centrifuged and collected, rinsed twice with washing buffer, and then incubated with 20 μM of fam-DEVD-fmk for caspase-3, fam-LETD-fmk for caspase-8, fam-LEHD-fmk for caspase-9 (Cell Technology Inc., Fremont, CA, USA), and FITC-ATAD-fmk for caspase-12 (Abcam Inc., Cambridge, UK) at 37 °C for 60 min, respectively. Labeled cells were analyzed by flow cytometry.

2.6. Caspase activity in selenite-induced apoptosis

HSC-3 cells were incubated with z-VAD-fmk (20 μM) or z-ATAD-fmk (20 μM) at 37 °C for 60 min, treated with 5 μM selenite for 24 h, and analyzed by TUNEL staining.

2.7. ER stress in selenite-induced apoptosis

HSC-3 cells were treated with 5 μM selenite for 24 h to measure the caspase-12 activity that is specifically associated with ER stress by flow cytometry, as described above. In addition, HSC-3 cells were treated with 10 μM selenite for 6 h and expressions of eIF-2α, GRP78, and GRP94 (which are specifically associated with ER stress) were analyzed by western blot. Briefly, selenite-treated cells were lysed in RIPA buffer (0.001% phenylmethylsulfonyl fluoride) and the cell lysates were centrifuged to collect the supernatant cellular proteins. Then, equal amounts of proteins were separated on SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. The membranes were blocked in Tris-buffered saline containing 5% non-fat milk and 1% Tween20 for 60 min. After blocking, the membranes were incubated with anti-β-actin (BioVision Inc., Milpitas, CA, USA), anti-BiP/GRP78 (Ana-Spec Inc., Fremont, CA, USA), anti-phospho-eIF2α (Ser^{51}) (CST Japan K.K., Tokyo, Japan), anti-total-eIF2α (CST Japan K.K.), and anti-HIF-1α (Japan BD Bioscience Inc.) at 4 °C overnight. Afterwards, the membranes were incubated with HRP-conjugated secondary sheep anti-mouse IgG (GE Healthcare Japan, K.K., Tokyo, Japan) or HRP-conjugated secondary goat anti-rabbit IgG (Santa Cruz Biotechnology Inc., Dallas, TX, USA). Blots were developed with chemiluminescence reagents (GE Healthcare Japan).

2.8. Effects of selenite on the PI3K/Akt pathway

HSC-3 cells were plated in 96-well tissue culture plates at a density of 2×10^4 cells per well and cultured for 24 h in a humidified atmosphere of 5% CO_2 at 37 °C in RPMI1640 with 10% fetal calf serum and 0.2% sodium hydrogen carbonate prior to incubation with 10 μM selenite or 30 μM LY294002. Twelve hours later, cellular levels of total Akt and phosphorylated Akt were measured using an ELISA kit (Active Motif Inc., Carlsbad, CA, USA) and estimated from the optical density at 450 nm, using a microplate reader. In addition, HSC-3 cells were treated with 10 μM selenite for 12 h, 30 μM LY294002 for 12 h, 5 μg/mL of CDDP for 24 h, 250 μg/mL of 5-FU for 48 h, 5 μg/mL of CDDP in the presence of 5 μM selenite for 24 h, or 250 μg/mL of 5-FU in the presence of 5 μM selenite for 48 h. After treatments, cellular proteins were collected and analyzed to evaluate the expression of Akt and phosphorylated Akt by western blot.

2.9. Statistical analysis

All data are presented as means ± standard deviation (SD) from three sets of independent experiments. Statistical significance of differences between means was calculated by Scheffé's test. A probability value of $p < 0.05$ was considered significant.

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