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Anticlastogenic and antigenotoxic effects of selenomethionine on doxorubicin-induced damage *in vitro* in human lymphocytes

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Abstract

The use of antioxidants during chemotherapy has been shown to reduce or prevent the undesirable effects experienced by healthy cells. Micronutrient selenium is well known for its antioxidant properties; however, selenium exhibits a bimodal nature in that both its beneficial and toxic properties lie within a limited and narrow dose range. The present study investigated the possible protective effects of selenomethionine (SM) on the cytotoxicity, genotoxicity and clastogenicity of the chemotherapic doxorubicin (DXR), a key chemotherapic used in cancer treatment. Human peripheral lymphocytes were treated *in vitro* with varying concentrations of SM (0.25 μM, 0.5 μM, 1.0 μM and 2.0 μM), tested in combination with DXR (0.15 μg/mL). SM alone was not cytotoxic and when combined with DXR treatment, reduced the DNA damage index significantly, the frequency of chromosomal aberrations, the number of aberrant metaphases and the frequency of apoptotic cells. The mechanism of chemoprotection of SM may be related to its antioxidant properties as well as its ability to interfere with DNA repair pathways. Therefore this study showed that SM is effective in reducing the genetic damage induced by the antitumoral agent DXR.

Keywords: Selenium; Selenomethionine; Doxorubicin; Comet assay; Chromosomal aberrations

1. Introduction

A large number of dietary components, such as vitamin C and E, lycopene and selenium are known for their antioxidant properties. Selenium is an essential micronutrient for animals, humans and microorganisms. A direct relationship between selenium intake and cancer risk in humans has been reported, indicating that selenium deficiency enhances the probability of developing cancer (Postovsky et al., 2003; Li et al., 2004).

However selenium, like some other trace elements, is bimodal in nature whereby its beneficial properties occur in a limited range of daily intake below which it cannot perform its essential functions, and above which it is toxic (Alaejos et al., 2000). As a result of these properties, selenium can be included in the class of ''Janus compounds'', having two 'faces' on the same head. It is well established that selenium participates in the process of detoxification by forming part of glutathione peroxidase, a cellular enzyme that maintains appropriately low levels of hydrogen peroxide within a cellular environment (Tapiero et al., 2003). Despite its antioxidant properties and requirement for human and animal nutrition, the appropriate form of selenium for supplementation continues to be debated. There exists a wide variety of selenium forms including sodium

Abbreviations: CAs, chromosomal aberrations; CT, chromatid breaks; DC, dicentric; DI, DNA damage index; DXR, doxorubicin; IC, chromosome breaks; MI, mitotic index; QR, quadriradial; R, ring; SM, selenomethionine; TR, triradial.

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selenite, sodium selenate, selenomethionine, selenocysteine and selenocystine. The L-isomer of selenomethionine (SM) is the most important natural food form of selenium found in seafood, grain products, meat and abundantly in Brazilian nuts (Alaejos et al., 2000; Schrauzer, 2000).

Some chemotherapeutic approaches have proposed the use of antioxidants to minimize cytotoxicity and the damage induced in normal tissues by antitumor agents that produce free radicals (Antunes and Takahashi, 1999). Although chemotherapy plays a major role in treating various cancers, especially in controlling advanced stages of malignancies in clinical settings, most cytotoxic chemotherapeutic agents used in the treatment of a wide range of cancers are not selective touching, neoplastic and health cells by its clastogenic effects (Ferguson and Pearson, 1996). These events may have important consequences in cancer chemotherapy since mutations may lead to drug resistance, limiting further therapy. Moreover, germ cell mutations may be transmitted to future generations and mutations may result in the development of secondary tumors from cells that were originally benign (Baguley and Ferguson, 1998). The chemotherapeutic doxorubicin (DXR), an anthracycline antibiotic commonly used to treat a wide variety of cancers (Quiles et al., 2002), produces an increase in DNA strand breakage, in the percentage of abnormal frequencies of chromosomal damage in the FISH and conventional chromosomal aberration assays and also increases micronucleus formation in human lymphocytes *in vitro* (Anderson et al., 1997; Dhawan et al., 2003), related to its activity when generating reactive oxygen species and inhibiting topoisomerase II complex (Gewitz, 1999).

Therefore, the present study was undertaken to investigate the possible antigenotoxic and anticlastogenic effects of selenomethionine (SM) in DNA damage induced by DXR in human lymphocytes *in vitro*. Damage was detected by assessing the mitotic index, chromosomal aberrations, comet assays and apoptotic cells.

2. Materials and methods

2.1. Cells and medium

Human peripheral blood was collected using heparinized vials from six healthy donors aged 26–30 years. Lymphocytes were isolated with plasma and cultured in RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 20% fetal calf serum (Cultilab, Campinas, SP, Brazil), penicillin (5 μg/mL), streptomycin (10 μg/mL) and 2% phytohemagglutinin (Life Technologies, Grand Island, NY). Cells were cultured at 37 °C in culture flasks containing 5 ml of complete medium.

The National Research Ethics Committee (process no. 8709/2001) approved the protocol of the experiments and written consent was obtained from each blood donor prior to joining the study.

2.2. Chemicals

L-Selenomethionine (CAS# 1464-42-2) was purchased from Acros Organics (New Jersey, USA) and DXR (CAS# 23214-92-8) (Laboratórios Eurofarma, São Paulo, Brazil) was donated from the Chemotherapy Center, Faculty of Medicine, USP, Ribeirão Preto. Both SM and DXR were dissolved in sterile distilled water prior to use.

2.3. Culture treatments

The concentrations of SM (0.25 μM, 0.5 μM, 1.0 μM and 2.0 μM) tested together with DXR were established in preliminary experiments; concentrations of SM above 2 μM were cytotoxic and reduced significantly mitotic index and cell viability (data not shown). The concentration of DXR (0.15 μg/mL) was similarly defined in preliminary experiments and besides the mitotic index, it was considered the number of chromosomal aberrations. In combined treatments (SM plus DXR) the values of mitotic index was similar to the negative control. The toxicity of the combined treatment is a critical factor because cytotoxic and cytostatic effects can mimic antimutagenicity because they interfere with the appearance of the mutant cells (Zeiger, 2007).

Three different types of SM treatment were assessed on DXR-induced damage cells. The cultures were treated with DXR 24 h after initiation of incubation and treatment with SM was performed either 2 h before, simultaneously, or 2 h after DXR treatment. After each respective treatment, DXR and SM remained in the cultures until harvesting.

Cells were harvested after 24 h of DXR treatment and then assessed for cell viability, apoptosis (apoptotic assay) and DNA damage of individual cells (comet assay), and after 26 h, for the analysis mitotic index and chromosomal aberrations, as DXR induces a delay in the cell cycle.

2.4. Comet assay

An aliquot of 300 μL from each culture was taken after 48 hours of incubation to test for cell viability by trypan blue exclusion and for the alkaline version of the Comet assay as described by Singh et al. (1988). Briefly, 300 μL of the cell suspension was centrifuged for 5 minutes (500 rpm) in a refrigerated microcentrifuge (Eppendorff). The resulting pellet was homogenized with 80 μL of a low melting point agarose (0.5%), spread onto microscope slides pre-coated with a normal melting point agarose (1.5%), and covered with a coverslip. After 5 min at 4 °C, the coverslip was removed and the slides were immersed in cold lysis solution (2.4 M NaCl; 100 mM EDTA; 10 mM Tris, 10% DMSO and 1% Triton-X, pH 10) for 24 h. After lysis, the slides were placed in an electrophoresis chamber and covered with electrophoresis buffer (300 mM NaOH per 1 mM EDTA, pH > 13), for a remaining 20 min to allow for unwinding of DNA. The electrophoresis proceeded for 20 min (25 V and 300 mA). Afterwards, the slides were submerged for 15 min in a neutralization buffer (0.4 M Tris–HCl, pH 7.5), dried at room temperature and fixed in 100% ethanol for 5 min. Slide staining was performed immediately before analyzing using ethidium bromide (20 μg/mL). Slides were prepared in duplicate and 100 cells were screened per sample (50 cells from each slide) in a fluorescent microscope (ZEISS, Germany) equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm using a 40× objective. The nucleus was classified visually according to the migration of the fragments in: class 0 (no damage); class 1 (little damage with a short tail length smaller than the diameter of the nucleus); class 2 (medium damage with a tail length one or two times the diameter of the nucleus); 3 (significant damage with a tail length between two and a half to three times the diameter of the nucleus); class 4 (significant damage with a long tail of damage greater than three times the diameter of the nucleus).

2.5. Metaphases preparation

To analyze chromosomal aberrations (CAs), a metaphase preparation was performed after 50 h of cell culture. Ninety minutes before harvesting, 12.5 μL colchicine (0.016%, Sigma) was added to each culture. The conventional cell harvest procedure was followed according to Moorhead et al. (1960). The cells were treated with hypotonic KCl solution (0.075 M) for 10 min, fixed with methanol:acetic acid (3:1), air-dried and stained with Giemsa: Sörensen buffer (1:30) for 5 min. All slides were coded. The same scorer analyzed the slides in a blind test and a total of 100 cells per