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Protective effect of β-glucan extracted from *Saccharomyces cerevisiae*, against DNA damage and cytotoxicity in wild-type (k1) and repair-deficient (xrs5) CHO cells

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Abstract

A large number of functional foods, including those that contain β-glucan, have been shown to prevent the development of cancer and other chronic diseases. The aim of the present study was to elucidate its mechanism of action, as well as to understand its effects as an antigenotoxic, anticlastogenic agent, and to determine its capacity to preserve cell viability. The investigation was carried out in the CHO-k1 and CHO-xrs5 cell lines. The cytokinesis-blocked micronucleus assay indicated that the different doses of β-glucan examined (5, 10, 20 and 40 μg/ml) did not show clastogenic effects. In the CHO-k1 cell line, a chemopreventive effect could be observed in all the protocols tested: pre-treatment (% reduction of 35.0–57.3), simultaneous treatment (simple – 5 reduction of 19.7–55.6 and with pre-incubation – of 42.7–56.4) and post-treatment (% reduction of 17.9–37.6). This finding indicates mechanisms of action involving desmutagenesis and bio-antimutagenesis, albeit the latter having a lesser role. However, in the repair-deficient CHO-xrs5 cells, β-glucan did not show a protective effect with post-treatment (% reduction of 2.96), thus supporting the involvement of bioantimutagenesis. The comet assay in CHO-k1 cells demonstrated that β-glucan has neither a genotoxic nor an antigenotoxic effect. Cell viability tests indicated that β-glucan preserves cell viability in both cell lines, preventing apoptotic events. These findings suggest that β-glucan, when present in foods, could provide them with nutraceutical characteristics and act as a dietary supplement, or that β-glucan could be used in new drug development.

Keywords: β-Glucan; CHO-xrs5 cells; CHO-k1; Comet assay; Cytokinesis-blocked micronucleus test

1. Introduction

Studies have pointed out that an increased intake of natural foods is directly related to a decreased risk for the development of cancer and other diseases (Weisburger, 2000; Ferrari and Torres, 2002). Therefore, investigations in toxicologic genetics are aimed at determining the genotoxic and clastogenic effects of xenobiotics, as well as to search for new substances that have chemoprotective effects or that can mitigate the adverse effects of harmful xenobiotics.

Among the components of these diets are some products that comprise the class of dietary fibers such as cereals, mainly oats and barley, mushrooms, algae and yeasts. In general, fibers can be sources of β-glucan. β-Glucan is one of the components present in large quantities in the cell wall of various organisms, and in the case of fungi it consists of a linear central skeleton of D-glucose molecules linked in the β-(1 → 3) position, containing side chains of various lengths that are made up β-1 → 6 linkages and that occur at different intervals along the central skeleton. Extraction provides a suspension of β-1,3 polyglucose particles (Di Luzio et al., 1979). The localization of this polysaccharide is the intermediate layer of the cell wall of yeasts which is

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adjacent to the plasma membrane, and its function is to maintain cell rigidity (Sandula et al., 1995).

The stimulation of the immune system against bacterial (Tzianabos and Cisneros, 1996), viral (Reynolds et al., 1980), fungal (Meira et al., 1996) and parasitic (Holbrook et al., 1981) infections; the modulation of humoral and cellular immunity (Falch et al., 2000); the stimulation of hematopoiesis (Hofer and Pospisil, 1997) and the activation of macrophages (Tsiapali et al., 2001) and neutrophils (Rankin et al., 1990) are some of the biological markers of different β-glucans.

Among other functions of β-glucan is its antigenotoxic capacity against DNA damage inducing agents, such as hydrogen peroxide (Slamenová et al., 2003), cyclophosphamide (Tohamy et al., 2003) and doxorubicin (Lin et al., 2004).

The *in vitro* micronucleus test is currently being used as a screening assay that can be performed using small concentrations of test article, which are often in limited supply at this stage and that has an easily analysis when compared to chromosome aberrations, thus requiring less time to make an assessment of the clastogenicity of a chemical (Garriot et al., 2002). The comet assay or single cell gel electrophoresis assay is a rapid and sensitive procedure for quantifying DNA lesions in individualized cells, both *in vitro* and *in vivo* (Tice et al., 1991; Fairbairn et al., 1995; Gontijo et al., 2001; Barbisan et al., 2003). The alkaline comet assay version was specially developed for detection of the DNA single-strand breaks and alkali-labile sites (Singh et al., 1988; Barbisan et al., 2003) and is also indicated to evaluate *in vivo* genotoxicity induced by carcinogen exposure (Anderson et al., 1998; Tsuda et al., 2000; Barbisan et al., 2003).

Even though the efficacy of β-glucan against these damages has been demonstrated, the mechanism of action of this substance has still not been totally elucidated, indicating the need for further studies. Thus, the aim of this study was to evaluate the mechanism of action of β-glucan extracted from *Saccharomyces cerevisiae*, where tests for antigenotoxicity, antimutagenicity and cell viability were conducted in Chinese hamster ovary cells (CHO), wild-type (k1) and DNA repair-deficient (xrs5). The methods used were the cytokinesis-blocked micronucleus assay, comet assay and cell viability based on differential staining with acridine orange and ethidium bromide. Different experimental protocols were employed: simple simultaneous treatment, simultaneous treatment after pre-incubation, pre-treatment and post-treatment.

2. Material and methods

2.1. DNA damage inducing agent

DNA damage was induced using the direct-acting, alkylating agent methylmethane sulfonate – MMS (Merck) diluted in sterile phosphate-buffered saline (PBS), Ca^{2+}- and Mg^{+2}-free, pH 7.4. The mechanism of mutagenesis for alkylating agents involves the transfer of a methyl or ethyl group to the nitrogenous bases of DNA, resulting in potential altered base pairing. In high concentrations, this agent also can be cytotoxic. The final concentration in the micronucleus assay was 37.6 μg/mL in the CHO-k1 line. However, for the CHO-xrs5 line a final concentration of 18.8 μg/mL was used. For the comet assay, a final concentration of MMS of 4.23 μg/mL was used in both lines. These doses were determined in pilot experiments.

2.2. Extraction of β-glucan

β-glucan, tested in this study, was extracted from *S. cerevisiae* and provided by Dr. Hevenilton José Matiazi, Laboratory of Technology of Foods and Medicines, Center for Food Sciences, Universidade Estadual de Londrina – PR.

The extraction of β-glucan with the main chains containing β-(1 → 3) linkages and lateral ramifications with β-(1 → 6) linkages was performed by autolysis of *S. cerevisiae*. The cell wall was separated by centrifugation at 6500 g for 8 min and heat-treated (70 °C for 5 h) in 10% NaOH, washed and centrifuged three times and dried in an oven at 40 °C. It was analyzed by NMR (chromatography by nuclear magnetic resonance), demonstrating the presence of (1,3 and 1,6) β-D-glucan with a purity of 85% and then dissolved in a dimethyl sufoxide (DMSO) and urea (8 M) solution (100 mL:60 g). In a warm water bath, 100 mL of DMSO with 10 mL of concentrated sulfuric acid was added, and the mixture was stirred for 4 h at 100 °C. The solution was dialyzed against approximately 100 L of ultra-pure water (Milli-Q) and then concentrated in a rotary evaporator at 40 °C followed by lyophilization.

The solution of the antimutagenic agent, β-glucan, was prepared in PBS and utilized at final concentrations in culture of 5, 10, 20 and 40 μg/mL, referred to in this work as β_1, β_2, β_3 and β_4, respectively. These doses were determined in pilot experiments.

2.3. Cell lines

Two Chinese hamster ovary (CHO) cell lines were used in this study: k1 wild-type cells and xrs5 cells deficient in the mechanism for repairing double-strand DNA. These two cell lines were chosen because the deficiency in repair of DNA damage in xrs5 cells could help proving the mechanism of action through bioantimutagenesis by β-glucan. They were both furnished by Dr. Catarina Satie Takahashi from the Mutagenesis Laboratory, Faculty of Medicine of Ribeirão Preto, Universidade Estadual de São Paulo. The micronucleus test was performed with cells grown in 5.0 mL of DMEM/F12 (Gibco) medium, supplemented with 10% fetal bovine serum (FBS, Gibco). Cells were cultivated as monolayers in 25-cm^2 flasks in a BOD-type incubator at 37 °C. In the comet assay, cells were grown in culture tubes with a flat surface (110 mm × 16 mm) containing 2.5 mL of culture medium according to the specifications described above. Under these conditions, the length of the cell cycle