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Antigenotoxicity of *Agaricus blazei* mushroom organic and aqueous extracts in chromosomal aberration and cytokinesis block micronucleus assays in CHO-k1 and HTC cells

M.F. Bellini [a], J.P.F. Angeli [a], R. Matuo [a], A.P. Terezan [b], L.R. Ribeiro [c], M.S. Mantovani [a,*]

[a] *Departamento de Biologia Geral, Universidade Estadual de Londrina—Campus Universitário, Cx. Postal 6001, 86051 990 Londrina, PR, Brazil*
[b] *Departamento de Química, Universidade Federal de São Carlos, 13565 905 São Carlos, SP, Brazil*
[c] *Departamento de Patologia, UNESP, 18600 000 Botucatu, SP, Brazil*

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Abstract

Agaricus blazei (Ab) has become popularly known for its medicinal properties. Scientifically, it has been tested with regard to its capacity to protect genetic material against damage. We examined different organic extracts (methanolic extract—ME, hexanic extract—HE and *n*-butanolic extract—BE) and an aqueous extract (AE) of Ab, for their capacity to induce DNA damage as well as for their protective effect. Genetic damage was determined by the chromosomal aberration assay (CA) in CHO-k1 cells for all extracts and the cytokinesis block micronucleus assay (CBMN) in non drug-metabolizing (CHO-k1) and drug-metabolizing (HTC) cell lines for extract BE only. The extracts did not show clastogenicity but showed anticlastogenicity. The greatest percent reduction obtained were with BE (105%) and AE (126%) treatments in CA. BE treatment did not display genotoxicity in CHO-k1, but was genotoxic in HTC. However, BE was shown to be antigenotoxic causing decreased micronucleus frequency in HTC and CHO-k1 cells. These results suggest that all the extracts contained protective substances, but in some cases they could show a genotoxic effect with regard to metabolism. Therefore, these findings warrant caution in the use of this mushroom by the population.

Keywords: Antigenotoxicity; *Agaricus blazei*; Drug metabolism; HTC cells; CHO-k1 cells; Chromosomal aberration assay

1. Introduction

Modern life exposes humans constantly to a large number of chemical, physical and biological agents. These agents can therefore interact in many ways with the human organism, and as a consequence act in a beneficial, neutral or harmful manner. Health problems and their solutions as a result of the above-mentioned agents' interactions have stirred interest among the scientific community.

Protective substances have been found in the diet, particularly in mushrooms including *Agaricus blazei* Murrill ss. Heinem. (Ab) (Lohman et al., 2001). *A. blazei* is a Brazilian mushroom popularly known as the sun mushroom, and it is frequently consumed as food or tea in different parts of the world, due to its medicinal effects. This mushroom is believed to fight physical and emotional stress, stimulate the immune system, improve the life quality in diabetics, reduce cholesterol, fight msosteoporosis and ulcers, treat circulatory and digestive problems, antitumor activity (Mizuno, 1995), anticarcinogenic properties (Takeda et al., 2000), antimutagenic and anticlastogenic effects

* Corresponding author. Fax: +55 43 3371 4527.
E-mail address: biomsm@uel.br (M.S. Mantovani).

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(Delmanto et al., 2001; Menoli et al., 2001; Oliveira et al., 2002; Bellini et al., 2003; Luiz et al., 2003a,b).

Kawagishi et al. (1989) were the first to separate the active compounds found in the Ab fruiting body. The authors detected polysaccharides with antitumor activity, the major fraction among these being FIII-2-b, which comprised a protein complex consisted of 43.3% protein, with (1 → 6)-β-D-glucan, and 50.2% carbohydrate. Polysaccharides composed of D-glucose chains with β-1,3 and β-1,6 linkages associated with antitumor activity act by stimulating the immune system, especially NK and macrophage cells (Itoh et al., 1994).

According to Wasser and Weiss (1999), the polysaccharides found in this basidiomycete could have important nutritional properties and many effects against the cancer development. Due to the large consumption of this mushroom in popular medicine, more data is needed on action mechanisms of its components. DNA damage are correlated with cancer development, therefore, the aim of the study was to determine the DNA-damaging potential and the antigenotoxic effects of *A. blazei* organic and aqueous extracts in a eukaryotic system in vitro with non drug-metabolizing (CHO-k1) and metabolizing (HTC) cells.

2. Material and methods

2.1. Preparation of Ab extracts

Ab extracts, strain 99/26, were kindly provided by Dr. Edson Rodrigues Filho and Ms. Ana Paula Terezan from the Chemistry Department of Universidade Federal de São Carlos. The fruiting body of the mushroom (896.90 g) was extracted with dichloromethane/hexane (50:50), dichloromethane (100), dichloromethane/methanol (50:50), methanol (100) and methanol/water (50:50). The hydroalcoholic extract obtained was first partitioned with dichloromethane, and the organic phase was concentrated and partitioned with methanol and hexane, yielding a hexanic fraction (HF/7.02 g) and methanolic fraction (MF/11.80 g). Subsequently, the hydroalcoholic extract was partitioned with ethyl acetate and *n*-butanol, respectively, yielding an aqueous fraction (AF/55.00 g) and an *n*-butanolic fraction (BF/16.93 g). These were dissolved in DMSO (dimethyl sulfoxide, Mallinckrodt) at a concentration of 5 mg/mL. Aliquots were stored frozen until use time. The final concentration in cultures was 100 μg/mL. This fraction were denominated: methanolic extract—ME, hexanic extract-HE, *n*-butanolic extract—BE and aqueous extract—AE.

2.2. DNA damage-inducing agents

DNA damage was induced in CHO-k1 cells using ethyl methanesulfonate (EMS, Acros), a direct-acting alkylating agent. A stock solution was prepared in sterile phosphate-buffered saline (PBS), Ca^{2+} and Mg^{2+} free, pH 7.4, and used at a final concentration of 310 μg/mL in cultures. 2-Aminoanthracene (2-AA, Acros), an indirect-acting alkylating agent, was used to induce DNA damage in HTC. The stock solution was prepared in dimethyl sulfoxide (DMSO, Mallinckrodt) and used at a final concentration of 1 μg/mL in cultures.

2.3. Cell lines

The Chinese hamster ovarian cell line CHO-k1 (wild-type) used in this study was provided by Dr. Catarina S. Takahashi in the Mutagenesis Laboratory, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo. HTC rat hepatoma cells derived from *Rattus novergicus* were acquired from the Rio de Janeiro Cell Bank. Cells were grown in DMEM/F-12 medium (Gibco), supplemented with 10% fetal bovine serum (FBS, Gibco), as monolayer in 25 cm^2 flasks in a BOD type incubator at 37 °C. Under these conditions, the cell cycle time was approximately 12 h for CHO-k1 and 24 h for HTC.

2.4. Chromosomal aberrations assay-CA

CHO-k1 cells were grown for one complete cell cycle (12 h) before treatments. The treatments were as follows: (a) control (solvent control, DMSO, 2%); (b) EMS (100 μg/mL); (c) ME (100 μg/mL); (d) HE (100 μg/mL); (e) BE (100 μg/mL); (f) AE (100 μg/mL); (g) ME combined with EMS; (h) HE combined with EMS; (i) BE combined with EMS; and (j) AE combined with EMS. The treatments were simultaneous and were carried out for one cycle. Colcemid (Demecolcine, Gibco) (0.1 μg/mL) was added at the treatments ending an hour before fixing. At harvest, the cells were trypsinized (0.025%) and then hypotonized in 1% sodium citrate solution at 37 °C for 20 min. The cells were fixed in methanol/acetic acid (3:1) and the slides were stained with 5% Giemsa for 5 min.

Three separate experiments were carried out for each treatment. A total of 300 metaphases were analyzed treatment type (100 cells/treatment/repetition). Chromosomal aberrations were classified as isochromatid (i.e. isochromatid breaks—ic; dicentric—dic; and ring—r) or chromatid (chromatid breaks—ct; triradial—tr, quadriradial—qr and complex rearrangement—cr) (Bez et al., 2001). The mitotic index (MI), corresponded to the number of cells in metaphase among 1000 cells analyzed per culture, was expressed as a mean percentage.

2.5. Cytokinesis block micronucleus assay—CBMN

Cells were grown for a complete cell cycle (12 h for CHO-k1 and 24 h for HTC) before treatments. The treatments were carried out as follows: (a) control (sol-