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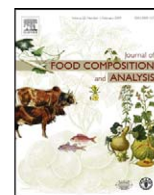

Original Article

Evaluation of the antigenotoxicity of polysaccharides and β-glucans from *Agaricus blazei*, a model study with the single cell gel electrophoresis/Hep G2 assay

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ABSTRACT

The mushroom *Agaricus blazei* (*Agaricus brasiliensis*) has been drawing attention because of its medicinal properties. Among its isolated compounds, special consideration is given to β-glucans, which are cell wall polysaccharides. The aim of the present work was to determine the genotoxic and/or antigenotoxic effects of the total polysaccharides of this mushroom and β-glucans, both extracted at different stages of fruiting body maturity (immature, mature stage with immature spores and mature stage with mature spores). β-glucan genotoxicity was examined using the comet assay in the HepG2 cell line. Additionally, the protective effect of total polysaccharides and β-glucans was tested against H_2O_2, bleomycin and doxorubicin. The results demonstrated that total polysaccharides and β-glucans had no genotoxic effects. On the contrary, they protected DNA against damage caused by the three inducers used. However, total polysaccharides had limited protective effects while being ineffective against doxorubicin. Interestingly, the largest protective effect was seen with extracts from the ripest stages and in the absence of isolated β-glucan.

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1. Introduction

Epidemiological studies have presented evidence that the occurrence of cancer in various organs such as stomach, colon, prostate and breast could be related to inappropriate diets (WCFR/IARC, 1997). The main factor involved in this process is related to the increase in mutation rate (Ferguson et al., 2005). Thus, a reduction in mutation rate in an organism could lead to a delay in the appearance of these neoplasms (Loeb et al., 2003). Introducing antimutagenic compounds into our diet would then be an attractive idea.

Among the products that arouse interest as a functional food is the mushroom *Agaricus blazei* (*Agaricus brasiliensis*) which is native to Brazil. The extract of this mushroom and isolated compounds have been widely studied due to their medicinal characteristics such as immune system stimulation, anticarcinogenic, antimutagenic and antioxidant properties (Kawagishi et al., 1989; Mizuno et al., 1990; Ohno et al., 2001; Dong et al., 2002).

Among the compounds isolated from the *A. blazei* are some polysaccharides with antitumor activity, including the β-glucans which have received much attention and which are credited with most of the mushroom's antitumor effects (Kawagishi et al., 1989; Mizuno et al., 1990; Ohno et al., 2001). Recently, we and others reported on the antimutagenic effect for β-glucan extracted from *A. blazei* (Angeli et al., 2006) and for β-glucans from different origins but having the same chemical structures (Chorvatovicová, 1993; Lazarova et al., 2004; Krizkova et al., 2006; Oliveira et al., 2007). A substantial part of this protective effect is due to an antioxidant property, where the compounds act mainly as reactive oxygen species (ROS) scavenger (Chorvatovicová, 1993; Lazarova et al., 2004). Thus, it is believed that this compound can be used as a chemopreventive agent, inhibiting the genotoxic actions of some harmful compounds.

It is important to emphasize that when extracting β-glucan from the fruiting body, the stage of maturity may influence the content and type of β-glucan found, which could result in great differences in biological responses (Camelini et al., 2005).

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Thus, the aim of the present work was to determine the genotoxic and/or antigenotoxic effects of total polysaccharides extracted at three different developmental stages of the mushroom (immature, SIa; mature with immature spores, SIIa; and mature with mature spores, SIIIa), with the total β-glucans extracted at these three different stages (SIb, SIIb and SIIIb) being tested as well. The findings of the study would help establish the best way of supplementation and/or harvesting time for nutraceutical purposes. Genotoxicity assessment was carried out in the human hepatoma cell line (Hep G2) which possesses a number of inducible phase I and phase II enzymes (Knasmüller et al., 1998) and has been used to investigate the chemoprotective effects of polysaccharides and β-glucan from *A. blazei* towards benzo(a)pyrene [B(a)P]-induced DNA damage in single cell gel electrophoresis (SCGE) assays—the Comet assay. Since these cells are of human origin, it is likely that they reflect the situation in humans better than other in vitro models (Knasmüller et al., 1998). Recently, Uhl et al. (1999, 2000) developed a protocol for the SCGE assay with HepG2 cells and found that this model can be used for the detection of genotoxic activities of different classes of genotoxic carcinogens.

2. Materials and methods

2.1. Extraction and purification of the polysaccharide fraction

Extraction and purification were performed as stated in Camelini et al. (2005), also chemical analysis is presented in the mentioned work.

2.2. DNA damage inducer

In the induction of DNA damage, three compounds were used for induction of DNA damage: H_2O_2 (100 μM, Nova Química), bleomycin (0.5 μg/mL, Sigma–Aldrich), and doxorubicin (1 μg/mL, Fluka); all compounds were diluted in Ca- and Mg-free PBS (phosphate-buffered saline).

2.3. HepG2 cell line and experimental protocols

The HepG2 cell line was kindly provided by Prof. Siegfried Knasmüller (Institute of Cancer Research, Medical University of Vienna). The cells were kept under liquid nitrogen and cultivated with Minimal Essential Medium (MEM), supplemented with 15% fetal bovine serum, both from Gibco (Paisely, Scotland), and 1% penicillin/streptomycin stock in 75 cm^2 flasks (TPP). Under such conditions, the duration of the cell cycle was approximately 24 h.

In the genotoxicity protocol, the cells were exposed to three different concentrations (5, 15, and 45 μg/mL, defined in previous experiments) of polysaccharide from the different stages of maturation for 24 h (concentrations determined in pilot experiments). As positive control, we used benzo(a)pyrene (10 μg/mL), and for negative control we used 1% dimethyl sulfoxide (DMSO).

Concerning the treatments of antigenotoxicity, the cells were exposed simultaneously to polysaccharides and to one of the different DNA damage inducers for three hours, in the case of bleomycin and doxorubicin, and for 5 min in ice for H_2O_2.

2.4. Single cell gel electrophoresis (SCGE)

With regard to the comet assay, we used the protocol described by Uhl et al. (1999, 2000) based on the premises proposed by Tice et al. (2000). In the experiments, the survival of the cells was determined with trypan blue (Lindl and Bauer, 1994), where only cultures with viability >80% were analyzed for comet formation. Briefly, at the end of the treatments, the cells were trypsinized (0.1% for 5 min), transferred to slides previously gelatinized, and lysed. After electrophoresis (25 V, 300 mA), the cells were stained with ethidium bromide (10 μg/mL, Sigma) and analyzed using a fluorescence microscopy (Nikon, model 027012).

Three independent repetitions were performed for each treatment. Regarding the comet assay, 100 cells were analyzed visually (Kobayashi et al., 1995), and classified according to the following criteria: (class 0) cells with undetectable damage—without tail; (class 1) cells with tails whose size was less than the diameter of the nucleus; (class 2) cells with tails whose size was from one to two times larger than the diameter of the nucleus; (class 3) cells with tails whose size was more than twice the diameter of nucleus. Afterward, the class value of each cell was summed to obtain the score (which ranged from 0 to 300); the mean and standard deviation was then calculated for each treatment. Apoptotic cells which had a totally fragmented nucleus were not considered in the analysis (Speit and Hartmann, 2005). The results obtained were evaluated using the Prism 4.0 statistical program for the Mann–Whitney test, which verified the difference among the treatments.

3. Results

The genotoxicity of total polysaccharides is depicted in Fig. 1. There was no alteration in cell viability (results not shown, considering optimal viability above 80%), nor was migration of DNA fragments as observed by the comet assay. The same can be observed in Fig. 2 which shows the results for only β-glucans. Similarly, there was no evidence of effects on cell viability (not shown) and DNA migration compared to control.

In testing for antigenotoxicty, β-glucan was combined with three DNA damage inducers. First, Fig. 3 presents the results of the combination with H_2O_2. In this case, the protective effect was noted at almost all concentrations of test substances, where smaller effects were observed with the total polysaccharides in the immature stage (SIa) and the greatest effects evidenced in the fraction containing only β-glucan in the mature stage (SIIb and SIIIb). The same response pattern was seen in the treatment with bleomycin (Fig. 4), showing a protective effect with the majority of the concentrations tested, but a reduced protective effect with total polysaccharides from the early stage as well as a more pronounced effect with the fraction containing only β-glucan in the ripe stage. When comparing the results (Figs. 3 and 4), a greater protection was seen against hydrogen peroxide because data positive control damage are greater to hydrogen peroxide treatment.

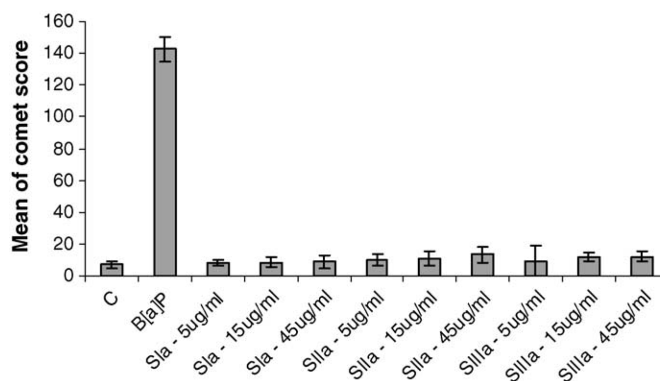

Fig. 1. Effects of polysaccharides from *Agaricus blazei* on DNA migration in HepG2 cell line. Bars indicate the mean of scores ± standard deviation of three independent repetitions (100 cells per slide were evaluated) (control DMSO, 1%; B[a]P, 5 μg/mL; SIa, polysaccharides extracted in unripe phase; SIIa, polysaccharides extracted in ripe but not sporulating phase; SIIIa, polysaccharides extracted in ripe sporulating phase).

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