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In vivo antimutagenic and antiatherogenic effects of the (1 → 3)(1 → 6)-β-D-glucan botryosphaeran

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ABSTRACT

The antimutagenic effect of botryosphaeran, an exocellular (1 → 3)(1 → 6)-β-D-glucan, from the ascomyceteous and plant-borne endophytic fungus, *Botryosphaeria rhodina* MAMB-05, was evaluated in young (6–8 weeks) and elderly (18 months) Swiss albino mice of both genders. The hypolipidemic, hypoglycemic and antiatherogenic potential was also evaluated in 18-month old male LDL receptor knockout (LDLr$^{-/-}$) mice. Administration of botryosphaeran by gavage (doses: 7.5, 15, 30 mg/kg b.w./day) in a 30-day pretreatment protocol (young mice), or 15-day protocol (older mice), did not cause genotoxicity as assessed by the micronucleus test in peripheral blood (PB) and bone marrow cells (BMCs). Furthermore, there was no cytotoxic effect of this β-D-glucan in the treatments. A lower frequency of micronuclei was observed in BMCs from young and old mice that received botryosphaeran, indicating its antimutagenic effect. Botryosphaeran (30 mg/kg b.w./day) promoted 102.22% (young) and 103.45% (elderly) reductions in cyclophosphamide-induced damage in male mice. Botryosphaeran also exerted chemoprotective effects in LDLr$^{-/-}$ and wild-type (C57BL/6) mice. Botryosphaeran treatment for 15 days at a dose of 30 mg/kg b.w./day improved the lipidic profile (reductions of 53.8–84.3%), and decreased aortic lipid deposition (32.8%) in the LDLr$^{-/-}$ atherosclerotic mice. The results indicate botryosphaeran has relevant biologic effects, making it a promising candidate for the development of new therapeutic agents.

1. Introduction

Over the past decades, there has been an increasing interest and consumption of natural medicinal plant and microbial products to alleviate or reduce the risks associated with cardiovascular disease, diabetes and cancer, which are responsible for significant morbidity and mortality [1]. Among these are macrofungal products such as mushrooms and brackets that have traditionally been used for millennia as folkloric medicines in the Orient to treat human disease conditions. They have also been recognized by the occidental world in clinical practice to treat cancers [2,3]. Among the bioactive compounds identified are the polysaccharides, and esp., the β-glucans that have been evaluated in animal models and cell lines [4]. These carbohydrate biopolymers act as biological response modifiers due to their

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immunomodulatory activities [5]. Other biological functions are also recognized and include antithrombosis, anti-inflammatory, anti-proliferation, hypolipidemia and hypoglycemia, and they also bear antioxidant properties [6,7]. Human clinical trials involving fungal polysaccharides have been conducted, but are few and the limited studies conducted were with small numbers of patients that were often poorly controlled [4].

The (1 → 3)(1 → 6)-β-D-glucan named botryosphaeran is an exocellular branched chain polysaccharide secreted by the fungus *Botryosphaeria rhodina* MAMB-05, and is composed of a main chain of (1 → 3)-linked β-D-glucose residues bound with a single branch comprising either glucose orgentiobiose linked by (1 → 6) bonds at every five glucose units along the backbone chain [8]. Botryosphaeran has been demonstrated from our research groups to possess a broad spectrum of activities with potential applications in the food, pharmaceutical and cosmetic sectors. For example, this mixed-linked β-D-glucan, has previously been shown in murine models that it lacked mutagenicity and exhibited strong anticlastogenic activity [9], and was demonstrated to possess hypoglycemic and hypocholesterolemic properties [10].

The antimutagenic and genotoxic, glucose and lipidic profiles and atheroprotective potential of botryosphaeran, however, has not been evaluated in a pre-treatment assay and experimental model ($LDLr^{-/-}$ mice) of atherosclerosis with 15-day and 30-day treatments using tests for genomic instability, and biochemical and morphologic parameters. Such studies are relevant because they may contribute to the development of innovative and promising strategies in the use of biomolecules to alleviate the effects of oxidative lesions in the genetic material that accompany the aging process and diseases, and to treat chronic degenerative diseases [11,12].

The objective of the present work was to investigate the (anti)genotoxic effect of botryosphaeran in Swiss albino mice of both genders, and of different age groups (young and elderly), as well as to assess its hypoglycemic, hypolipidemic, and antiatherogenic potential in older male mice (WT-C57BL/6 and $LDLr^{-/-}$ knockout) with a predisposition to atherosclerosis.

2. Material and methods

2.1. Microorganism and culture conditions

B. rhodina (isolate MAMB-05), an ascomyceteous endophytic fungus, was grown in Erlenmeyer flasks by submerged fermentation on sucrose as sole carbon source for 72 h at 28 °C under shaking conditions (180 rpm) as previously described [8].

2.2. Botryosphaeran production and solution preparation

After cultivating the fungus, the mycelium was removed by centrifugation (1250 × *g*/15 min), the supernatant was recovered, and the exopolysaccharide was precipitated from the solution by adding three volumes of isopropanol and leaving the solution stand overnight at 4 °C. The precipitate was recovered by centrifugation, dissolved in distilled water (gently heated at 60 °C for 2 h) and dialyzed exhaustively for 48 h, with frequent changes of water. The botryosphaeran-containing solution was then lyophilized and the dried powder was stored at −20 °C until use. For use in the *in vivo* assays, botryosphaeran was solubilized in isotonic saline solution (0.9%, w/v) at a concentration of 3 g/L (stock solution). A sample of this solution was used to quantify total sugars by the phenol-sulfuric acid method [13], and to confirm the concentration of the stock solution. The doses of botryosphaeran were chosen based on its solubility limit of 3 g/L in isotonic saline solution [9].

2.3. Experimental design

The experiments were conducted in strict accordance with the recommendations of the ethics principles and guidelines of the National Institutes of Health (USA) for the care and handling of laboratory animals. This study was approved by the Research Ethical Committee on Animal Use at Universidade Federal do Espírito Santo (CEUA/UFES case 017/2013). For the use and handling of experimental inbred animals (wild-type C57BL/6 and $LDLr^{-/-}$ knockout mice), the standards established by Comissão Técnica Nacional de Biossegurança (Brazil) were followed.

2.3.1. Experimental protocol to assess antimutagenic and anticytotoxic activities of botryosphaeran in young and aged mice

2.3.1.1. Animals and treatments. Swiss albino mice (*Mus musculus*) were obtained from Biotério do Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, and housed in groups in standard plastic cages in a room set with constant temperature (22–23 °C), relative humidity (50 ± 10%), and a light-dark cycle of 12-h under fluorescent lighting. The animals received the classic standard commercial animal ration (Nuvilab CR1, Nuvital, Colombo-PR, Brazil) and water *ad libitum*.

Fifty young mice aged 6–8 weeks (25 males and 25 females) and 50 older mice aged 18 months (25 males and 25 females) were preconditioned on the standard commercial animal ration for one week prior to the start of the treatments.

Botryosphaeran was evaluated using the pre-treatment protocol in the following experimental groups: (i) botryosphaeran-treated groups with administered doses of 7.5, 15 and 30 mg/kg animal body weight (b.w.), (ii) positive control group treated with cyclophosphamide (Sigma-Aldrich, St. Louis, MO, USA), and (iii) negative control group treated with isotonic saline. Each experimental group consisted of 10 animals that were randomly selected and separated by gender. Young mice had body weights ranging from 39.5 ± 4.0 g (males) to 35.7 ± 1.4 g (females), and the aged mice had body weights ranging from 50.4 ± 3.4 g (males) to 42.1 ± 3.0 g (females).

The pre-treated groups of Swiss albino mice received each dose of botryosphaeran by gavage (7.5, 15 and 30 mg botryosphaeran/kg animal b.w.) once daily for 30 days for the young animals, and for 15 days for the older animals. At the end of each of the treatment periods with the three doses of botryosphaeran by gavage, the clastogenic agent cyclophosphamide was administered intraperitoneally to the young animals at a dose of 100 mg/kg b.w., and to the older animals at a dose of 50 mg/kg b.w. These doses and the different time intervals for treatments (15 and 30 days) were defined according to previous tests. The negative control group received isotonic saline solution by gavage, whereas the positive control group received only 100 and 50 mg/kg b.w. (intraperitoneally) to induce micronucleus formation, according to the age category of the animals.

Aged (18- month old) male, low-density lipoprotein-receptor knockout ($LDLr^{-/-}$) mice (LDL receptor-deficient mice show elevated plasma cholesterol levels and develop atherosclerosis on feeding a lipid-rich diet), which had a C57BL/6 (wild-type, WT) genetic background, were obtained from the Laboratórios de Fisiopatologia de Doenças Humanas e Animais, of Universidade Vila Velha (Vila Velha, Espírito Santo), and Fisiologia Translacional of Universidade Federal do Espírito Santo. The animals were maintained in the latter vivarium in groups housed in standard plastic cages under the same temperature, relative humidity and light-dark cycle conditions mentioned above, and were fed the classic standard commercial animal ration and water *ad libitum*.

When the $LDLr^{-/-}$ animals reached 18 months of age, they received a Western-type diet (Rhoster®, Araçoiaba da Serra, São Paulo, Brazil) to accelerate spontaneous hyperlipidemia and the development of atherosclerotic lesions. The composition of this atherogenic diet is shown in Table 1. After five weeks (18–month old animals plus five weeks), the $LDLr^{-/-}$ mice were divided into three groups: (i) animals that were treated with botryosphaeran (30 mg/kg b.w.) by gavage once