Toxicology in Vitro 36 (2016) 18–25

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Review

Chemopreventive effect and lack of genotoxicity and mutagenicity of the exopolysaccharide botryosphaeran on human lymphocytes

M. Malini [a,*,1], M.S. Camargo [b], L.C. Hernandes [c], C.G. Vargas-Rechia [d], E.A. Varanda [b], A.M. Barbosa [e,2], R.F.H. Dekker [e], S.T. Matsumoto [f], L.M.G. Antunes [c], I.M.S. Cólus [a]

[a] Departamento de Biologia Geral, Centro de Ciências Biológicas - Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Km 380, CEP 86.057-970, Londrina, , PR, Brazil
[b] Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas de Araraquara - Universidade Estadual Paulista Júlio de Mesquita Filho, Rodovia Araraquara-Jaú, Km 01, s/n, CEP 14.800-903, Araraquara, SP, Brazil
[c] Departamento de Análises Clinicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Av. do Café, s/n, CEP 14.040-903, Ribeirão Preto, SP, Brazil
[d] Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Av. do Café, s/n, CEP 14.040-903, Ribeirão Preto, SP, Brazil
[e] Biorefining Research Institute, Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada
[f] Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais - Universidade Federal do Espírito Santo, Avenida Fernando Ferrari, 514, CEP 29.075-910, Vitória, ES, Brazil

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 20 June 2016
Accepted 30 June 2016
Available online 5 July 2016

Keywords:
Ames test
Comet assay
(1 → 3)(1 → 6)-β-D-glucan
Jurkat cells
Human T lymphocytes
Antigenotoxicity

ABSTRACT

Carbohydrate biopolymers of fungal-origin are an important natural resource in the search for new bioagents with therapeutic and nutraceutical potential. In this study the mutagenic, genotoxic, antigenotoxic and antioxidant properties of the fungal exopolysaccharide botryosphaeran, a (1 → 3)(1 → 6)-β-D-glucan, from *Botryosphaeria rhodina* MAMB-05, was evaluated. The mutagenicity was assessed at five concentrations in *Salmonella typhimurium* by the Ames test. Normal and tumor (Jurkat cells) human T lymphocyte cultures were used to evaluate the genotoxicity and antigenotoxicity (Comet assay) of botryosphaeran alone and in combination with the mutagen methyl methanesulfonate (MMS). The ability of botryosphaeran to reduce the production of reactive oxygen and nitrogen species (RONS) generated by hydrogen peroxide was assessed using the CM-H_2DCFDA probe in lymphocyte cultures under different treatment times. None of the evaluated botryosphaeran concentrations were mutagenic in bacteria, nor induced genotoxicity in normal and tumor lymphocytes. Botryosphaeran protected lymphocyte DNA against damage caused by MMS under *simultaneous treatment* and *post-treatment* conditions. However, botryosphaeran was not able to reduce the RONS generated by H_2O_2. Besides the absence of genotoxicity, botryosphaeran exerted a protective effect on human lymphocytes against genotoxic damage caused by MMS. These results are important in the validation of botryosphaeran as a therapeutic agent targeting health promotion.

Contents

* Corresponding author at: Departamento de Biologia Geral - CCB, Universidade Estadual de Londrina – UEL, Rodovia Celso Garcia Cid, PR (445), Km 380, CEP 86057-970 Londrina, Paraná, Brazil.
E-mail addresses: maressa.malini@gmail.com (M. Malini), marianasantoro@gmail.com (M.S. Camargo), livia.hernandes@yahoo.com.br (L.C. Hernandes), cvrechia@fcfrp.usp.br (C.G. Vargas-Rechia), eavaranda@gmail.com (E.A. Varanda), anelibarbosa@gmail.com (A.M. Barbosa), rdekker@lakeheadu.ca (R.F.H. Dekker), siltamie@gmail.com (S.T. Matsumoto), lusania@fcfrp.usp.br (L.M.G. Antunes), ilcecolus@gmail.com (I.M.S. Cólus).
[1] Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais - Universidade Federal do Espírito Santo, Avenida Fernando Ferrari, 514, CEP 29.075-910, Vitória, ES, Brazil.
[2] Departamento de Química, Centro de Ciências Exatas - Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR 445, Km 380, CEP 86.057-970, Londrina, PR, Brazil.

http://dx.doi.org/10.1016/j.tiv.2016.06.008
0887-2333/

1. Introduction

Carbohydrate biopolymers (polysaccharides) of fungal-origin are an important natural resource in the search for new agents with therapeutic potential. Among them are the β-glucans, notably the (1 → 3)(1 → 6)-β-D-glucans, that are known to modify biological responses (Bohn and BeMiller, 1995), and play roles in chemoprevention, and can manifest immunomodulatory and antitumor activities, among others (Kagimura et al., 2015; Kirkland et al., 2011). β-D-Glucans of the (1 → 3)-type are not digested by enzymes such as the β-(1 → 3)-glucanases, which are absent from the gastrointestinal tract of mammals. Thus, they survive longer in the tissues of mammals, and can therefore be a candidate bioagent in treating cancers (Miura et al., 1996). Their ability to non-specifically activate the cellular and humoral components of the host immune system is well documented in the literature, see for example, (Williams, 2009). Accordingly, this activation leads to the increased functional activity of immune cells such as macrophages, mononuclear cells and neutrophils (Liang et al., 1998). Despite the studies that show the absence of toxicity of these compounds and their derivatives (Kagimura et al., 2015; Miranda-Nantes et al., 2011; Miranda et al., 2008; Roupas et al., 2012; Weng et al., 2011), the evidence of the absence of risk to health and the mechanism by which the (1 → 3)-β-D-glucans exert their effects on mammalian cells are key to their validation as bioactive agents.

The exopolysaccharide (EPS) botryosphaeran is of the (1 → 3)(1 → 6)-β-D-glucan type and is secreted by the fungus, *Botryosphaeria rhodina* MAMB-05. Botryosphaeran consists of a linear backbone chain of D-glucose residues linked by (1 → 3)-β-glucosidic bonds, with side-branches comprising glucose and gentiobiose linked through (1 → 6)-β-glucosidic bonds (Barbosa et al., 2003). It is soluble in water and forms gels and viscous solutions that may favor its commercial applications in different sectors such as health, biotechnology, food, pharmaceutical and cosmetology (Kagimura et al., 2015).

Studies from our research groups have evaluated the biological effects of botryosphaeran. This (1 → 3)(1 → 6)-β-D-glucan (i) did not present mutagenic activity (micronucleus test) in mice, and exhibited strong antimutagenic activity (Miranda et al., 2008); (ii) promoted hypoglycemic activity reducing the levels of plasma glucose in diabetes-induced rats by 52%, and reduced LDL-cholesterol levels in hyperlipidaemic-conditioned rats by 18% (Miranda-Nantes et al., 2011); (iii) exerted an antiproliferative effect in breast cancer MCF-7 cells that was associated with apoptosis, necrosis and oxidative stress (Queiroz et al., 2015); (iv) in combination with the antineoplastic agent, doxorubicin, botryosphaeran was selective for leukemic T lymphocyte cells (Malini et al., 2015). The molecular mechanisms of action of botryosphaeran appear to be involved in the repression of genes related to the G1 phase of the cell cycle (Malini et al., 2015). When derivatized by sulfonylation, the sulfonated biopolymer exhibited anticoagulant and antithrombotic activities (Brandi et al., 2011). In addition, botryosphaeran was found to possess free radical scavenging properties and antioxidant activity (Giese et al., 2015). In another study with a different *B. rhodina* strain (RCYU 30101), botryosphaeran demonstrated the ability to activate the lymphoblastogenesis process (Weng et al., 2011).

Understanding the mechanisms by which botryosphaeran exerts its chemoprotective effect is important in validating this β-glucan as a therapeutic agent. Studies on botryosphaeran's ability to inhibit the genotoxic effect caused by several mutagens are key to understanding its mechanism of action. The antimutagenic activity consists of the peculiar ability of a substance to reduce the frequency of spontaneous or induced mutations regardless of the mechanism involved (Von Borstel et al., 1996). Słoczyńska et al. (2014) recently proposed that various classes of compounds can be distinguished, such as compounds with antioxidant activity; compounds that inhibit the activation of mutagens; blocking agents; as well as compounds characterized with multiple mechanisms of action.

It is necessary and important to use *in vitro* tests employing normal and tumor cells in order to rapidly assess the selectivity of botryosphaeran as a therapeutic agent, given its biological effects, and the potential applications of this EPS described in the literature by our research group.

In the present study the objectives were to investigate: (i) the effects of the treatment with botryosphaeran alone, or in combination with the mutagen, methyl methanesulfonate (MMS), on normal and tumor (Jurkat cells) human T lymphocytes in order to evaluate its genotoxic and chemoprotective effects on these cell types; (ii) the possible mutagenicity of botryosphaeran assessed by the Ames test on different *Salmonella typhimurium* strains; and (iii) the antioxidant effect against H_2O_2-induced production of RONS (reactive oxygen and nitrogen species) on normal and tumor lymphocytes.

2. Material and methods

2.1. Microorganism and culture conditions

Botryosphaeria rhodina (MAMB-05 isolate) was grown by submerged fermentation (SmF) on sucrose as sole carbon source for 72 h at 28 °C as described by Steluti et al. (2004).

2.2. Production of botryosphaeran

Following the growth of the fungus, the mycelium was removed by centrifugation (1250g/15 min) and the supernatant recovered to which was then added three volumes of isopropanol, and left to stand overnight at 4 °C. The precipitate was recovered by centrifugation, resolubilized in water with gentle heating, and then dialyzed exhaustively against water for 48 h at 4 °C. Thereafter, the dialyzed solution