Anticlastogenic effect of β -glucan, extracted from *Saccharomyces cerevisiae*, on cultured cells exposed to ultraviolet radiation

Ariane Fernanda da Silva · Rodrigo Juliano Oliveira ·
Andressa Megumi Niwa · Gláucia Fernanda Rocha D'Epiro ·
Lúcia Regina Ribeiro · Mário Sérgio Mantovani

Received: 11 October 2011 / Accepted: 28 February 2012 / Published online: 9 June 2012
© Springer Science+Business Media B.V. 2012

Abstract β -glucan is an important polysaccharide due to its medicinal properties of stimulating the immune system and preventing chronic diseases such as cancer. The aim of the present study was to determine the anticlastogenic effect of β -glucan in cells exposed to ultraviolet radiation (UV). Chromosome aberration assay was performed in drug-metabolizing cells (HTC) and non drug-metabolizing cells (CHO-K1 and repair-deficient CHO-xrs5), using different treatment protocols. Continuous treatment (UV + β -glucan) was not effective in reducing the DNA damage only in CHO-xrs5 cells. However, the pre-treatment protocol (β -glucan before UV exposition) was effective in reducing DNA damage only in CHO-K1 cells. In post-treatment (β -glucan after UV exposition) did not show significative anticlastogenic effects, although there was a tendency toward prevention. The data suggest that β -glucan has more than one action mechanism, being

capable of exerting desmutagenic as well as bio-antimutagenic action. The findings also suggest that the presence of the xenobiotic metabolizing system can reduce the chemopreventive capacity of β -glucan. Therefore, these results indicate that β -glucan from *Saccharomyces cerevisiae* can be used in the prevention and/or reduction of DNA damage.

Keywords β -Glucan · Anticlastogenicity · Ultraviolet radiation · Cell culture

Introduction

There are some substances which can interact with the genetic material, constituting a potential risk to human health. In this manner, alterations of the human genome resulting from human activity (occupation, life style, diet), have led to a preoccupation with adopting protective measures for future generation.

Therefore, various studies have been carried out with the aim of identifying antimutagenic substances, since they can also be anticarcinogenic. Anticarcinogenic substances are capable of impeding, retarding or reducing the development or progression of tumors. These substances can act by three pathways: inhibiting the formation of mutagenic and/or carcinogenic agents; inhibiting the transfer of the carcinogen to specific cells (called blocking agents); and inhibiting the expression of malignant characteristics (called suppressor agents; Kuroda and Hara 1999).

A. F. da Silva · A. M. Niwa · G. F. R. D'Epiro (✉) ·
M. S. Mantovani
Departamento de Biologia Geral, Universidade Estadual
de Londrina, Campus Universitário, Londrina, Paraná,
Brazil
e-mail: glaucia_depiro@hotmail.com

R. J. Oliveira
Universidade Federal de Mato Grosso do Sul, Campo
Grande, Mato Grosso do Sul, Brazil

L. R. Ribeiro
Departamento de Biologia Celular, Universidade Estadual
Paulista, Rio Claro, Brazil

Thus, many diseases can be prevented by controlling the exposure of humans to mutagenic substances. Epidemiologic, in vivo and in vitro studies have shown an inversely proportional association in the consumption of fruits and vegetables with the risk of developing cancers or other diseases (Ferrari 2001; Ferrari and Torres 2002; Flagg et al. 1995; Weisburger 1999, 2000; Zhang et al. 1999).

Functional foods not only satisfy basic nutritional needs but also provide beneficial physiologic effects (Riezzo et al. 2005). Among these effects, notable ones are reduction in cholesterol, antioxidant and anticarcinogenic properties, and prevention of type 2 diabetes (Arvanitoyannis and Van Houwelingen-Koukaliaroglou 2005; Riccardi et al. 2005). β -glucans are polysaccharides present in the cell walls of microorganisms (bacteria and fungi) and plants (barley and oats; Cisneros et al. 1996; Zimmerman et al. 1998).

β -glucans have demonstrated anti-hypercholesterolemic, anti-hyperglycemic, anti-hypertriglyceridemic, anti-arteriosclerotic and anti-diabetic properties (Kim et al. 2005). In addition, they act stimulating the immune system of the host, thereby exerting a beneficial effect against a variety of infections (Tzianabos and Cisneros 1996). Also, β -glucans have been described as modulators of both humoral and cellular immunity (Kubala et al. 2003; Takahashi et al. 2001) and as stimulators of growth and differentiation of bone marrow cells (Lin et al. 2004).

This polysaccharide has also stirred the interest of investigators in the area of cancer, due to various studies that have demonstrated antigenotoxic, antimutagenic and antioxidative properties (Angeli et al. 2006; Krizkova et al. 2003; Mantovani et al. 2008; Oliveira et al. 2006, 2007; Slameňova et al. 2003; Tohamy et al. 2003) against the induction of damage to the genetic material by various chemical agents. This study investigated the anticlastogenic activity of β -glucan against DNA damage induced by ultraviolet radiation in HTC (drug-metabolizing), CHO-K1 (non drug-metabolizing) and CHO-xrs5 (non drug-metabolizing and repair deficient) cells.

Materials and methods

Cell lines and culture conditions

The hepatoma cell line of *Rattus norvegicus* (HTC), utilized in the present work, was acquired from the

Cell Bank of the Federal University of Rio de Janeiro (UFRJ). Chinese hamster ovarian cell lines used were wild-type CHO-K1 and CHO-xrs5 which is deficient in the repair of double-strand DNA, both supplied by the Mutagenesis Laboratory of the School of Medicine of Ribeirão Preto, State University of São Paulo (USP).

The cells were grown in DMEM/F12 medium (Gibco) supplemented with 10 % fetal bovine serum (Gibco) in BOD type incubator, at 37 °C. The cells were cultivated in 25-cm² flasks as monolayer. Under these conditions, the cell cycle is approximately 24 h for HTC and 12 h for CHO-K1 and CHO-xrs5.

DNA damage-inducing agent

DNA damage was induced by ultraviolet light for an exposure time of 5 s. The luminous intensity was 20 μ W/cm², and the exposure time was determined in pilot experiments.

Chemopreventive agent

The β -glucan tested in this study was extracted from *Saccharomyces cerevisiae* and donated by Dr. Hevenilton Jose Matiazi of the Laboratório de Tecnologia de Alimentos e Medicamentos, Universidade Estadual de Londrina. The solution of β -glucan was prepared in sterile Ca²⁺- and Mg²⁺-free PBS, pH 7.4, and utilized concentration of 40 μ g/mL in culture.

Chromosomal aberration assay

Cells maintained in 25 cm² flasks were trypsinized and the trypsin was inactivated with complete medium. A drop of the cell suspension was placed in Neubauer chamber for cell counting. The number of cells present in five diagonal squares was determined and the mean was multiplied by 25×10^4 , which furnished the number of cells in 1.0 mL of cell suspension. A total of 1.0×10^6 cells were transferred to each well of 6-well cell culture plates along with 5.0 mL of complete medium. The plates were placed in an incubator to allow the cells to grow for one cell cycle (24 h for HTC and 12 h for CHO-K1 and CHO-xrs5). After this period, cells were exposed to ultraviolet radiation by placing the plates under the ultraviolet light in the laminar flow hood for 5 s. The cells were returned to the incubator for another cell

cycle period, and afterward harvested, guaranteeing that they would undergo at least one cell cycle after induction of damage to the genetic material, since the aberrations can be lost in subsequent cell divisions. Cells were harvested after 2 h additional exposure to colcemide (0.05 $\mu\text{g/mL}$) added to the medium.

The cells were harvested with 0.025 % trypsin–EDTA followed by hypotonization with sodium citrate (1 %), and fixation was carried out with methanol–acetic acid (3:1). The slides were stained with 5 % Giemsa in Sorensen buffer (pH 7.0) for 5 min, washed in running water, air-dried and conditioned in the refrigerator until analysis, which was performed with a light microscope at 100 $\times$ magnification. The analysis was performed by examining 100 metaphases in each culture. The structural alterations observed were: gaps (chromatid and chromosomal), breaks (chromatid and chromosomal), ring, dicentric chromosomes, triradial figures, quadriradial figures, acentric fragments and complex rearrangements. The metaphases that showed more than ten aberrations were classified as multiple aberrations.

Treatment protocols

The cell lines HTC and CHO-k1 were submitted to the following experimental protocols: (a) negative control: protected from UV light by a plate lid and a Kraft paper; (b) positive control: exposed to UV; (c) β -glucan: cells were seeded with 40 $\mu\text{g/mL}$ β -glucan and remained until cell harvest being protected from UV light; (d) pre-treatment: cells were seeded with 40 $\mu\text{g/mL}$ β -glucan and later they were

washed with PBS before UV light exposition; (e) continuous treatment: cells were seeded with 40 $\mu\text{g/mL}$ β -glucan and remained during UV light exposition; and (f) post-treatment: 40 $\mu\text{g/mL}$ β -glucan was added after UV light exposition and remained until cell harvest. Investigation in CHO-xrs5 cell line was performed only in post-treatment protocol. All the experiments were carried out in triplicate.

Percent reduction in DNA damage and statistical analysis

The percent reduction in chromosome aberrations was calculated by dividing the number of cells with aberrations found with the inducing agent minus the number of cells with aberrations found with the anticlastogenic treatments by the number of cells with aberrations found with the damage-inducing agent minus the number of cells with aberrations found in the control, multiplied by 100 (Waters et al. 1990). Statistical analysis for the chromosomal aberration assay was carried out using the Chi-squared test ($p < 0.05$).

Results

The total number of aberrations, the frequency of aberrations per cell and the types of chromosomal aberrations observed in the anticlastogenic protocols with β -glucan in HTC cells exposed to UV light are presented in Table 1. Table 2 presents the total number of metaphases with aberrations, the mean

Table 1 Total number of aberrations, the frequency of aberrations per cell and the types of chromosomal aberrations observed in the anticlastogenic protocols with β -glucan in HTC cells exposed to UV light

Protocols	Treatment	Total of Aberrations	Aberrations per cell	Types of Aberrations								Gaps		MA
				dic	ib	cb	qd	tr	cr	ring	af	is	ct	
–	Control	20	1.18	2	0	2	3	3	0	3	0	3	4	0
–	UV	54	1.10	8	3	15	5	1	3	1	0	6	12	1
–	β G	24	1.33	4	1	8	1	0	4	0	0	3	3	0
Anticlastogenicity														
Pre-treatment	β G + UV	69	1.44	14	1	37	5	0	3	2	0	1	6	0
Continuous	β G + UV	24	1.14	5	0	9	0	0	2	0	0	1	7	1
Post-treatment	UV + β G	39	1.22	7	1	19	2	0	2	0	0	2	6	0

UV ultraviolet radiation, β G β -glucan 40 $\mu\text{g/mL}$, dic dicentric, ib isocromatidic break, cb cromatidic break, qd quadriradial, tr triradial, cr complex rearrange, af acentric fragment, is isochromatidic, ct chromatidic, MA multiple aberrations

and standard deviation of the frequency of aberrations and the percent reduction in damage by β -glucan in HTC cells. The statistical analysis of these data demonstrates that β -glucan does not show clastogenic activity. The anticlastogenic protocols demonstrated that there was no efficacy with pre and post-treatments which showed a percent reduction in damage of 3.12 and 53.12 %, respectively. However, the treatment protocol with continuous exposure to β -glucan showed a large reduction in damage, about 87.5 %, which was statistically significant.

Table 3 demonstrates the total number of aberrations, the frequency of aberrations per cell and the types of chromosome aberrations observed in the

protocols for anticlastogenicity with β -glucan in CHO-K1 cells exposed to UV light. The total number of metaphases with aberrations, the mean and standard deviation of the frequency of aberrations and the percent reduction in damage with β G in CHO-K1 cells can be seen in Table 4. It is shown again that β -glucan did not exert a clastogenic effect. In this type of cell, unlike with HTC, the pre-treatment protocol was effective in reducing the frequency of chromosome aberrations, as with continuous treatment. Despite that post-treatment with β -glucan showed a reduction in the induction of DNA damage by UV light, this difference was not statistically significant, when compared with the control. The percent reduction in damage for the

Table 2 Total number of metaphases with aberrations, the mean and standard deviation of the frequency of aberrations and the percent reduction in damage by β -glucan in HTC cells

Protocol	Treatment	Cells with aberrations		
		Total of metaphases with aberrations	Mean $\pm$ standard deviation	Percent reduction of the damage (%)
–	Control	17	5.67 $\pm$ 0.58	–
–	UV	49 ^{a*}	16.33 $\pm$ 4.62	–
–	β G	18 ^a	6.00 $\pm$ 1.00	–
Anticlastogenicity				
Pre-treatment	β G + UV	48 ^b	16.00 $\pm$ 5.57	3.12
Continuous	β G + UV	21 ^{b*}	7.00 $\pm$ 2.64	87.50
Post-treatment	UV + β G	32 ^b	10.67 $\pm$ 0.58	53.12

UV ultraviolet radiation, β G β -glucan 40 μ g/mL

* Statistically significant

^a Control comparison

^b UV comparison

Table 3 Total number of aberrations, the frequency of aberrations per cell and the types of chromosomal aberrations observed in the anticlastogenic protocols with β -glucan in CHO-K1 cells exposed to UV light

Protocols	Treatment	Total of aberrations	Aberrations per cell	Types of Aberrations								Gaps		MA
				dic	ib	cb	qd	tr	cr	ring	af	is	ct	
–	Control	17	1.06	7	3	1	0	0	0	0	0	4	2	1
–	UV	37	1.09	14	0	6	0	0	1	1	1	9	5	0
–	β G	10	1.43	3	4	1	0	0	1	1	0	0	0	0
Anticlastogenicity														
Pre-treatment	BG + UV	13	1.00	3	3	2	1	0	0	0	0	2	2	0
Continuous	β G + UV	18	1.00	2	6	5	0	0	0	0	1	4	0	1
Post-treatment	UV + β G	21	1.05	8	3	2	0	0	0	0	0	7	1	0

UV ultraviolet radiation, β G β -glucan 40 μ g/mL, *dic* dicentric, *ib* isochromatidic break, *cb* chromatidic break, *qd* quadriradial, *tr* triradial, *cr* complex rearrange, *af* acentric fragment, *is* isochromatidic, *ct* chromatidic, *MA* multiple aberrations

pre-, post- and continuous treatment protocols was 116.67, 77.78 and 88.89 %, respectively.

Table 5 presents the data obtained in CHO-xrs5 cells using the continuous treatment protocol, with regard to the total number of aberrations, frequency of aberrations per cell and types of chromosome aberrations observed. The total number of metaphases with aberrations, the means and standard deviation of the frequency of aberrations found in the CHO-xrs5 cells, as well as the percent reduction in DNA damage by β -glucan are shown in Table 6. Statistical analysis demonstrates that β -glucan was not clastogenic and that continuous treatment was not effective in preventing damage caused by ultraviolet radiation, with only 18.75 % damage reduction.

Discussion

The effects of sunlight have fascinated researchers for decades because nearly every living organism on earth is likely to be exposed to sunlight, including its UV fraction (Shimizu 2010), and all biological cells are rich in UV-absorbing agents such as nucleic acids and proteins (Sinha and Häder 2002). The first and main target-structure of UV radiation in animals is the body surface, including the skin and eyes (Vargas et al. 2011). Meanwhile, ultraviolet light is a potent mutagen for unicellular organisms and for cultured cells grown as monolayer.

In this study, efficacy of β -glucan in reducing the clastogenic damage resulting from exposure to UV

Table 4 Total number of metaphases with aberrations, the mean and standard deviation of the frequency of aberrations and the percent reduction in damage by β -glucan in CHO-K1 cells

Protocol	Treatment	Cells with aberrations		
		Total of metaphases with aberration	Mean $\pm$ standard deviation	Percent reduction of the damage (%)
–	Control	16	5.33 $\pm$ 1.15	–
–	UV	34 ^{a*}	11.33 $\pm$ 0.58	–
–	β G	7 ^a	2.33 $\pm$ 2.31	–
Anticlastogenicity				
Pre-treatment	β G + UV	13 ^{b*}	4.33 $\pm$ 2.08	116.67
Continuous	β G + UV	18 ^{b*}	6.00 $\pm$ 2.00	88.89
Post-treatment	UV + β G	20 ^b	6.67 $\pm$ 3.06	77.78

UV ultraviolet radiation, β G β -glucan 40 μ g/mL

* Statistically significant

^a Control comparison

^b UV comparison

Table 5 Total number of aberrations, the frequency of aberrations per cell and the types of chromosomal aberrations observed in the anticlastogenic protocols with β -glucan in CHO-xrs5 cells exposed to UV light

Protocol	Treatment	Total of aberrations	Aberrations per cell	Types of aberrations								Gaps		MA
				dic	ib	cb	qd	tr	cr	ring	af	ic	ct	
–	Control	6	1.20	2	0	1	0	0	3	0	0	0	0	0
–	UV	23	1.10	2	1	10	0	0	2	0	0	0	8	0
–	β G	7	1.00	1	0	2	0	0	1	0	0	1	2	0
Anticlastogenicity														
Continuous	β G + UV	25	1.39	3	2	11	0	0	5	0	0	1	3	0

UV Ultraviolet radiation, β G β -glucan 40 μ g/mL, *dic* dicentric, *ib* isochromatidic break, *cb* chromatidic break, *qd* quadriradial, *tr* triradial, *cr* complex rearrange, *af* acentric fragment, *is* isochromatidic, *ct* chromatidic, *MA* multiple aberrations

Table 6 Total number of metaphases with aberrations, the mean and standard deviation of the frequency of aberrations and the percent reduction in damage by β -glucan in CHO-xrs5 cells

Protocol	Treatment	Cells with aberrations		
		Total of metaphases with aberrations	Mean $\pm$ standard deviation	Percent reduction of the damage (%)
–	Control	5	1.67 $\pm$ 1.15	–
–	UV	21 ^{a*}	7.00 $\pm$ 1.73	–
–	β G	7 ^a	2.33 $\pm$ 0.58	–
Anticlastogenicity				
Continuous	β G + UV	18 ^b	6.00 $\pm$ 1.00	18.75

UV Ultraviolet radiation, β G β -glucan 40 μ g/mL

* Statistically significant

^a Control comparison

^b UV comparison

light was shown in the protocol of continuous treatment, for the HTC cell line. This chemopreventive action was shown to be very significant, as it produced a reduction in damage of about 87.5 %. However, the protocols of pre-treatment and post-treatment did not show evidence of anticlastogenic effects.

Basically, there are two classes of DNA protective substances, those involving a desmutagenic mechanism and those a bio-antimutagenic mechanism (Kada et al. 1982). Desmutagenic substances are those capable of impeding the action of damage-inducing agents, principally by adsorbing these potentially genotoxic chemicals, thereby acting preferentially in the extracellular medium. However, bio-antimutagenic agents play a role in the prevention of DNA lesions or in their repair, acting inside the cell (Kada and Shimoi 1987). De Flora (1998) noted that bio-antimutagenic substances also act by modulating DNA repair and replication, or by stimulating error-free repair of damage to DNA, or by inhibiting repair systems subject to error. However, reports with respect to these activities against physical agents are not found in the pertinent literature.

Studies with chemical agents indicated that the protocol of continuous treatment can indicate desmutagenic as well as bio-antimutagenic activity. Thus, the two protocols of pre- and post-treatments would be necessary to help in the differentiation of these two types of mechanisms.

In accordance with these findings, it is possible that β -glucan possesses both desmutagenic and bio-

antimutagenic activity, in view of its statistically significant efficacy in the protocol of continuous treatment.

Studies that relate the mechanism of action of anticlastogenic agents against the induction of DNA damage by physical inducers are only recent. However, one could suppose that β -glucan forms a protective barrier for the cell at the moment of exposure to UV light. As non-ionizing radiations have a low penetrating capacity, ultraviolet rays, for example, would not have sufficient energy to overcome this barrier, which would impede the excitation of electrons in the more outer shells of the atoms of nitrogenous bases. This fact would explain the high level of chemoprevention afforded by continuous treatment and would confirm the hypothesis of desmutagenesis.

The present study then corroborates the hypothesis of desmutagenesis proposed by Slameňova et al. (2003) who investigated the protective effect of three different extracts of glucan, at three different concentrations, against damage induced by hydrogen peroxide and methylene blue in the presence of visible light. This study demonstrated that pre-treatment with the glucans was effective in preventing genotoxic effects detected by the comet assay.

The lack of effect in the pre-treatment protocol in the present study can be explained by the possibility that β -glucan in the culture medium was metabolized, considering that HTC cells are proficient in metabolizing xenobiotics and considering that any unmetabolized β -glucan was removed from the culture

medium before exposure to UV light, which would have negated the formation of any protective barrier for the cells, leaving them more susceptible to the action of ultraviolet radiation.

The hypothesis of bioantimutagenesis cannot be ruled out, since the post-treatment protocol, despite not showing statistically significant difference, produced a percent reduction in damage of 53.12 %.

When analyzing the results obtained with the CHO-K1 cell line, it was seen again that continuous treatment was effective in reducing damage caused by UV light. It was also demonstrated that, unlike with the HTC line, pre-treatment with β -glucan reduced the frequency of chromosomal aberrations found in CHO-K1 cells, showing it to be effective in reducing the frequency of such aberrations even to baseline levels.

Therefore, it is seen that the hypothesis of interference in metabolism in the chemopreventive effect of β -glucan is pertinent, since the protective effect was demonstrated in the pre-treatment of the non drug-metabolizing cell line and not of the drug-metabolizing line. An important factor in this regard that should be taken into consideration is the time of exposure of the cells to β -glucan, since the HTC cells were exposed to this polysaccharide for a period twice as long as that for the CHO-K1 cells.

The repair-deficient cell line CHO-xrs5 did not demonstrate a chemoprotective effect in the protocol tested, continuous treatment, showing a percent reduction in damage of only 18.75 %. This finding supports the hypothesis of bioantimutagenesis, albeit at a lower efficacy, since the lack of repair mechanisms influenced the response to treatment with β -glucan.

The hypothesis that β -glucan acts through desmutagenesis as well as bioantimutagenesis, albeit less effectively, is supported by the works of Oliveira et al. (2006, 2007), in which β -glucans of different origin were tested, *Saccharomyces cerevisiae* and barley, against the effects of DNA damage-inducing chemical agents, namely methylmethane sulfonate and 2-aminoanthracene, using treatment protocols of simultaneous simple and pre-incubation, pre- and post-treatment.

In addition to the desmutagenesis and bioantimutagenesis hypothesis, β -glucan can act as an antioxidant, as described by Slameňova et al. (2003). They demonstrated that β -glucan was capable to sequester free radicals generated by exposure to methylene blue in the presence of visible light and to hydrogen peroxide.

Therefore, based on the principle that cancer can be induced by a mutational event (Ames et al. 1973) and taking into account the chemopreventive character of β -glucan, one could consider the utilization of β -glucans as anticarcinogenic and/or antimutagenic agents, in the prevention of DNA damage caused by physical as well as chemical agents, such as ultraviolet radiation. The fact that β -glucan did not show any clastogenic damage in any of the cell lines tested allows us to suggest that its consumption is safe, since it is present in various functional foods that comprise part of the diet of the population. However, new studies are necessary to determine the actual mechanisms of action of β -glucan, to optimize the forms of administration of β -glucan in a possible dietary supplementation.

Acknowledgments This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Fundação Araucária. We also thank Dr. A. Leyva for English language editing of the manuscript.

References

- Ames BN, Durston WE, Yamasaki E, Lee FD (1973) Carcinogens are mutagens. A simple test system combining liver homogenates for activation and bacteria for detection. *Natl Acad Sci USA* 70:2281–2285
- Angeli JPF, Ribeiro LR, Gonzaga MLC, Soares SA, Ricardo MPNS, Tsuboy MS, Stidl R, Knasmüller S, Linhares RE, Mantovani MS (2006) Protective effects of β -glucan extracted from *Agaricus brasiliensis* against chemically induced DNA damage in human lymphocytes. *Cell Biol Toxicol* 22:285–291
- Arvanitoyannis IS, Van Houwelingen-Koukiliaroglou M (2005) Functional foods: a survey of health claims, pros and cons, and current legislation. *Crit Rev Food Sci Nutr* 45:385–404
- Cisneros RL, Gibson FC, Tzianabos AO (1996) Passive transfer of poly-(1–6)- β -glucotriosyl-(1–3)- β -glucopyranose glucan protection against lethal infection in an animal model of intra-abdominal sepsis. *Infect Immun* 64:2201–2205
- De Flora S (1998) Mechanisms of inhibitors of mutagenesis and carcinogenesis. *Mutat Res* 402:151–158
- Ferrari CKB (2001) Oxidative stress pathophysiology searching for an effective antioxidant protection. *Int Med J* 8:175–184
- Ferrari CKB, Torres EAFS (2002) New dietetic compounds with anticarcinogenic properties. *Rev Bras Canc* 48: 375–382
- Flagg EW, Coates RJ, Greenberg RS (1995) Epidemiologic studies of antioxidants and cancer in humans. *J Am Coll Nutr* 14:419–427
- Kada T, Shimoi K (1987) Desmutagens and bio-antimutagens: their modes of action. *Bioessays* 7:113–116

- Kada T, Inoue T, Namiki N (1982) Environmental desmutagens and antidesmutagens. In: Klekowski EJ (ed) Environmental mutagens and plant biology. Praeger, New York, pp 137–151
- Kim YW, Kim KH, Choi HJ, Lee DS (2005) Anti-diabetic activity of β -glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*. Biotechnol Lett 27:483–487
- Krizkova L, Durackova Z, Sandula J, Slamenova D, Sasinkova V, Sivonova M, Krajcovic J (2003) Fungal beta-(1–3)-D-glucan derivatives exhibit high antioxidative and antimutagenic activity in vitro. Anticancer Res 23:2751–2756
- Kubala L, Ruzickova J, Nickova K, Sandula J, Ciz M, Lojek A (2003) The effect of (1 → 3)- β -D-glucans, carboxymethylglucan and schizophyllan on human leukocytes in vitro. Carbohydr Res 338:2835–2840
- Kuroda Y, Hara Y (1999) Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res 436:69–97
- Lin H, She Y, Cassileth B, Sirotak F, Rundles SC (2004) Maitake beta-glucan MD-fraction enhances bone marrow colony formation and reduces doxorubicin toxicity in vitro. Int Immunopharmacol 4:91–99
- Mantovani MS, Bellini MF, Angeli JPF, Ribeiro LR (2008) β -glucans in promoting health: prevention against mutation and cancer. Mutat Res 658:154–161
- Oliveira RJ, Ribeiro LR, Silva AF, Matuo R, Mantovani MS (2006) Evaluation of antimutagenic activity and mechanisms of action of β -glucan from barley, in CHO-K1 and HTC cell lines using the micronucleus test. Toxicol In Vitro 20:1225–1233
- Oliveira RJ, Matuo R, Silva AF, Matiazi HJ, Mantovani MS, Ribeiro LR (2007) Protective effect of beta-glucan extracted from *Saccharomyces cerevisiae*, against DNA damage and cytotoxicity in wild-type (k1) and repair deficient (xrs5) CHO cells. Toxicol In Vitro 21:41–52
- Riccardi G, Capaldo B, Vaccaro O (2005) Functional foods in the management of obesity and type 2 diabetes. Curr Opin Clin Nutr Metab Care 8:630–635
- Riezzo G, Chiloiri M, Russo F (2005) Functional Foods: Salient Features and Clinical Applications. Immune Endocr Metabol Disord 5:331–337
- Shimizu T (2010) The role of macrophage migration inhibitory factor (MIF) in ultraviolet radiation-induced carcinogenesis. Cancers 2:1555–1564
- Sinha RP, Häder D-P (2002) UV-induced DNA damage and repair: a review. Photochem Photobiol Sci 1:225–236
- Slameňova D, Labaj J, Krizkova L, Kogan G, Sandula J, Bressgen N, Eckl P (2003) Protective effects of fungal (1 → 3)- β -D-glucan derivatives against oxidative DNA lesions in V79 hamster lung cells. Cancer Lett 198:153–160
- Takahashi H, Ohno N, Adachi Y, Yadomae T (2001) Association of immunological disorders in tethal side effect of NSAIDs on β -glucan-administered mice. FEMS Immunol Med Microbiol 31:1–14
- Tohamy AA, El-Ghor AA, El-Nahas SM, Noshay MM (2003) β -Glucan inhibits the genotoxicity of cyclophosphamide, adramycin and cisplatin. Mutat Res 541:45–53
- Tzianabos AO, Cisneros RL (1996) Prophylaxis with the immunomodulator PGG glucan enhances antibiotic efficacy in rats infected with antibiotic-resistant bacteria. Ann NY Acad Sci 797:285–287
- Vargas MA, Geihs MA, Maciel FE, Cruz BP, Nery LEM, Allodi S (2011) The effects of UV radiation on the visual system of the crab *Neohelice granulata*: a protective role of melatonin. Comp Biochem Physiol C Toxicol Pharmacol 154:427–434
- Waters MD, Brady AL, Stack HF, Brockman HE (1990) Antimutagenicity profiles for some model compounds. Mutat Res 238:57–85
- Weisburger JH (1999) Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. Food Chem Toxicol 37:943–948
- Weisburger JH (2000) Eat to live, not live to eat. Nutrition 16:767–773
- Zhang S, Hunter DJ, Forman MR, Rosner BA, Speizer FE, Colditz GA, Manson JE, Hankinson SE, Willett WC (1999) Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. J Natl Cancer Inst 91:547–556
- Zimmerman JW, Lindermuth J, Fish PA, Palace GP, Stevenson TT, Demong DE (1998) A novel carbohydrate-glycosphingolipid interaction between a β -(1–3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. J Biol Chem 273:22014–22020