Regulatory Toxicology and Pharmacology 53 (2009) 164–173

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Effects of the polysaccharide β-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice

Rodrigo Juliano Oliveira [a], Maria José Sparça Salles [a,*], Ariane Fernanda da Silva [a], Tatiane Yumi Nakamura Kanno [a], Ana Carolina dos Santos Lourenço [a], Gabriele Antico Freiria [a], Hevenilton José Matiazi [b], Lúcia Regina Ribeiro [c], Mário Sérgio Mantovani [a]

[a] Department of Biology, Center of Biological Sciences, Londrina State University, Londrina, PR, Brazil
[b] Laboratory of Food and Drugs Technology, Londrina State University, Londrina, PR, Brazil
[c] Department of Biology, State University of São Paulo, Rio Claro, SP, Brazil

ARTICLE INFO

Article history:
Received 6 September 2008
Available online 31 December 2008

Keywords:
β-Glucan
Teratogenicity
Cyclophosphamide
Mutagenic

ABSTRACT

Alterations that could lead to the cancer development may also be related to an adverse development of offspring in experimental animals. Some functional foods, which contain the polysaccharide β-glucan, have been described as being effective in the prevention of clastogenic damage. Based on that finding, the aim of the present study was to determine the efficacy of this sugar polymer in the mutagenic and teratogenic damage control. Two sets of females, pregnant and non-pregnant, were evaluated. The results indicated that β-glucan was effective in preventing clastogenic damage in pregnant as well as non-pregnant females. In addition, pregnant females were more susceptible to mutagenic damage. However, teratogenic effects were not prevented effectively, although there was a trend toward a reduction in level of malformations. Despite β-glucan did not prevent malformations, it increased fetal viability and reduced number of post-implantation losses and resorption, thereby enhancing reproductive performance in females.

1. Introduction

Mutagenic and teratogenic events are related, since both result from permanent alterations attached in DNA. These alterations produce changes in gene expression that could lead to adverse phenotypes, as a result of alterations mostly on mitotic events involved on the growth process, morphogenesis and cellular differentiation in regard to the maturation of the physiological processes (Lewin, 2001).

Genetic factors are the most common cause of congenital malformations, being responsible for about one third of cases in humans (Moore and Persuad, 2004). Estimates on the incidence of primary congenital causes of malformations point to chromosomal aberrations as responsible for 6–7% of the cases, mutant genes for 7–8%, environmental factors for 7–10%, multifactorial heredity for 20–25% and unknown etiology for 50–60% (Connor and Ferguson-Smith, 1987; Persaud, 1990; Thompson et al., 1991). However, it is possible that majority of children with unknown congenital malformation etiologies has some genetic disturbance (Moore and Persuad, 2004).

Besides the association between mutagenic and teratogenic events, it can be inferred that both are related with other events to induce cancer development. Since several carcinogens can interact with genetic material, the mutagenicity and the carcinogenicity might be related. There is enough information proving that cancer is derived from a mutational event, by activating a proto-oncogene, stimulating the cellular proliferation, or inactivate a tumor-suppressor gene, thus failing in repressing cellular division. Both events previously related are essential to change gene expression for the conversion of a cell in a transformed phenotype. Many oncogenes act in routes that lead the alteration of gene expression ultimately (Lewin, 2001).

As mutagenicity, teratogenicity and carcinogenicity are related, it is believed that increased growth of industrial, agricultural, domestic and urban residues from anthropogenic activities accounts for the increase on such events in human populations. Therefore, there is an increasing need for genetic tests to measure potential risks to public health and to detect natural and/or synthetic agents with attributes to reduce and/or to avoid harmful effects of the causing substances (Ferrari, 1991).

Since various studies have indicated a proportionally inverse association between consumption of fruits and vegetables and development of chronic diseases such as cancer (Ferguson, 1994; Flagg et al., 1995), substances present in such a diet should be

* Corresponding author. Fax: +55 43 3371 4527.
E-mail address: mazecisv@gmail.com (M.J.S. Salles).

0273-2300/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2008.12.007

tested to determine their relevance in the prevention of mutagenic and/or teratogenic effects.

Some studies have pointed the capacity of β-glucan to prevent clastogenic and genotoxic damage due to cyclophosphamide, cisplatin, doxorubicin and hydrogen peroxide (Slamenová et al., 2003; Tohamy et al., 2003; Lin et al., 2004). However, there are no prior reported studies on the β-glucan's utilization in preventing teratogenicity. β-Glucan is extracted from *Saccharomyces cerevisiae* cells by autolysis. It is a D-glucose's polymer that contains in its linear skeleton central linkages in the β-(1→3) position. This molecule also contains branching β-(1→6) linkages (Di Luzio et al., 1979).

Cyclophosphamide is a DNA damage-inducing and teratogenic agent. That chemotherapeutic and immunosuppressive agent is activated mainly in the liver and its metabolites have a potent mutagenic and teratogenic effect.

The objectives of the present work were to evaluate the effect of β-glucan on reproductive performance of females exposed to cyclophosphamide, as well as its capability of preventing teratogenesis mediated by that chemotherapeutic. Besides, it was verified the anticlastogenic activity of β-glucan on pregnant and no-pregnant females, to infer on a possible correlation between prevention of clastogenic and teratogenic damages.

2. Materials and methods

2.1. DNA damage-inducing and teratogenic agent

Cyclophosphamide (Sigma), an indirect-acting alkylating agent was used to induce DNA damage and teratogenesis. It was prepared in phosphate-buffered saline (PBS) and administered at a dose of 20 mg kg^{-1} (intraperitoneal, i.p.).

2.2. Preparation of β-glucan

β-Glucans was extracted from *S. cerevisiae* cells by autolysis according to Matiazi (2006) and administered at a dose of 150 mg kg^{-1} body weight (i.p.).

2.3. Animals and experimental design

Male and female Swiss mice (*Mus musculus*) from our own colony, weighing 30 g, were used. The animals were housed in polypropylene cages with wood shavings as bedding, kept under controlled temperature, with a 12:12 h light: dark schedule and free access to food and tap water. All experiments were performed in accordance with the NIH Principles of Laboratory Animal Care.

Sixty female and 20 males were randomly divided into two lots. The first comprised 40 animals which were submitted to overnight crossing, at a proportion of 1 male to 2 females, for determination of teratogenicity, mutagenicity and antimutagenicity. Pregnancy was determined by the copulation plug detection in the morning after overnight breeding, in which day was considered gestation zero day. The second lot with 20 non-pregnant females was used to study mutagenicity and antimutagenicity.

2.4. Teratogenicity assay

The first set of mice, pregnant females ($n = 40$), was subdivided into 4 experimental groups ($n = 10$ each). The animals of the control group (group 1) received sterile PBS in a 0.1 ml 10 g^{-1} body weight's volume, intraperitoneal (i.p.), on the 9–11th gestation day. The animals in the cyclophosphamide group (group 2) received this cytotoxic agent at a dose of 20 mg kg^{-1} body weight (i.p.) on the 10th gestation day. The animals in the β-glucan group (group 3) received this polysaccharide on the 9–11th day of gestation at a dose of 150 mg kg^{-1} body weight (i.p.). The animals belonging to the combined treatment group (group 4) received β-glucan at 150 mg kg^{-1} body weight (i.p.) on the 9–11th gestation's day, and 20 mg/kg body weight (i.p.) of cyclophosphamide on the 10th gestation day.

The chosen dose and day for the cyclophosphamide were based on literature (Chernoff et al., 1989), in which the 10th gestation's day is considered to be of a larger spectrum for malformations resulting from the acute administration of cyclophosphamide (Francis et al., 1990). The β-glucan dose and protocol were established according to the description of the mechanism's action of that polysaccharide (Tohamy et al., 2003; Oliveira et al., 2006, 2007).

The gestation period was continued until the 18th day, when the females were killed by cervical dislocation, followed by laparotomy. The corrected maternal weight gain (maternal weight gain minus gravid uterus weight) was determined and the visceral organs were inspected for macroscopic abnormalities. Next, the lungs, heart, liver and kidneys were removed and weighed. Then, a hysterectomy and umbilectomy were performed, recording the number of implantation sites, presence of resorptions, number of live and dead fetuses, and fetal and placental weights. A systematic analysis was also performed to detect external malformations and determine sex. Based on these data, the following fertility parameters were determined: resorption's level (no. of resorptions × 100/no. of implantations); post-implantation losses' level (no. of implantations–no. of live fetuses × 100/no. of implantations); sex ratio (no. of male/no. of female); placental index (placental weight/fetal weight); external malformations' level (no. of malformed fetuses × 100/no. of fetuses examined).

Correction of weight for gestational age was determined based on the Calderon's method (Calderon, 1988), in which fetuses can be classified as: fetuses of adequate weight for gestational age (AWGA) – mean weight ± SD of fetuses in the control group; fetuses of low weight for gestational age (LWGA) – body weight below the SD's lower limit of the control fetuses' mean weight; and high weight's fetuses for gestational age (HWGA) – body weight above the SD's upper limit of the control fetuses' mean weight.

The offspring group was divided randomly into two subgroups each consisting of half the litter. The first was fixed in Bodiańs solution for visceral examination which was performed using the incisions/microdissection proposed by Barrow and Taylor (1969) for the thorax and abdomen's study, and using the strategic incisions proposed by Wilson (1965) for the head's study. The classification of visceral alterations was based mainly on the works of Taylor (1986) and Manson and Kang (1994) and alterations proposed by Oliveira (2001). The second subgroup was reserved for skeletal examination according to the Alizarin red technique described by Staples and Schnell (1964). The examination of visceral and skeletal fetuses was performed using a dissecting stereomicroscope. The comparison of quantitative results was carried out using parametric and non-parametric tests (ANOVA, Kruskal–Wallis and χ^2), depending on the nature of the data distribution. For the qualitative data and frequencies, the litter was utilized as the unit basis, as recommended in the literature (Manson et al., 1982; Hanseman and Hogan, 1995). However, for the quantification of the visceral and skeletal malformations the fetus was used as basic unit according to Moreira et al. (2005). In all cases, differences with $p < 0.05$ were considered statistically significant.

2.5. Micronucleus assay in peripheral blood

In the first lot of the experimental groups, peripheral blood was collected by tail vein puncture at four different times to determine mutagenicity and antimutagenicity. Blood samples designated by