Radiation Physics and Chemistry 78 (2009) 433–436

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

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

Immune-enhancing activities of low molecular weight β-glucan depolymerized by gamma irradiation

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ARTICLE INFO

Keywords:
β-glucan
Gamma irradiation
Immune activity
Cytokine
Macrophage

ABSTRACT

β-glucans are structural cell wall polymers of many microorganisms and cereals which possess immunomodulatory properties and have been used in the food, cosmetic and medical industry. In our previous study, β-glucan was depolymerized by gamma irradiation and leads to improve the solubility and viscosity. This study was carried out to evaluate the functional properties, mainly immune-enhancing activities of low molecular weight β-glucan fragmented by gamma irradiation. The results showed that RAW 264.7 macrophage cell stimulation activities of irradiated β-glucan were higher than that of non-irradiated β-glucan. In addition, the oral administration of gamma-irradiated β-glucan significantly increased the proliferation and cytokine (IFN-γ and IL-2) release of spleen and Peyer's patch cells compared with non-irradiated β-glucan. In conclusion, gamma irradiation could be used as an effective method for the production of depolymerized β-glucan improved functional property such as immunomodulatory activity.

1. Introduction

β-glucan is composed of glucose units linked together to form a long polymer chain and is a fiber-type derived from the cell walls of yeast, oat, barley and many medicinal mushrooms (Brown and Gordon, 2003; Wood, 1986; Yamada, 2000). These, β-glucan has immune-enhancing activities, which nutritionally potentiate and modulate an immune response (Borchers et al., 2004; Falch et al., 2000). It is known that the immunomodulatory effects of β-glucan are influenced by its degree of branching, polymer length and tertiary structure, but it is still difficult to confidently make generalizations because of the often contradictory data available (Borchers et al., 2004; Estrada et al., 1997).

It has been reported that the depolymerization method by using acidic (Hasegawa et al., 1993; Bohn and BeMiller, 1995) or enzymatic hydrolysis (Shimokawa et al., 1996) has been used to improve the physical and functional properties of β-glucan. Although these methods are effective in decreasing the molecular weight, they do have certain disadvantages such as a high cost, low yield, long processing time and acidic wastes from a chemical or enzymatic treatment (Jeon and Kim, 2002; Marie et al., 1994).

Gamma irradiation is used for a final biological sterilization (Hugo, 1995) of materials that can be subsequently used for manufacturing biomedical products. In addition, ionizing radiation leads to the degradation of polysaccharides such as starch, cellulose and pectin by the cleavage of the glycosidic bonds (Charlesby, 1981; Cho et al., 2003; Dauphin and Saint-Lébe, 1977; Sokhey and Hanna, 1993). The basic advantages of degradation of polymers by radiation include the ability to promote changes reproducibly and quantitatively, without the introduction of chemical reagents and without the need for a special equipments/setup to control the temperature, environment and additives (Charlesby, 1981). Our previous study (Byun et al., 2008) reported that gamma irradiation could be used as an effective method to produce a low molecular weight β-glucan with high solubility and low viscosity. However, the effects of irradiation on the physiological activities of β-glucan have not been evaluated.

The purpose of this study was to compare the immunomodulatory activities of non-irradiated and irradiated β-glucan by *in vitro* and *in vivo* studies on the proliferation and cytokine release of macrophage, spleen and Peyer's patch cells.

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0969-806X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.radphyschem.2009.03.022

2. Materials and methods

2.1. Sample preparation and gamma irradiation

β-glucan was purchased from Ace Biotech, Ltd. (Chungbuk, Korea), which was purified from the black yeast, *Aureobasidium* sp. Powdered β-glucan was dissolved in deionized water to obtain a concentration of 100 mg/ml (10%, w/v), and the solution was stored at 4 °C before irradiation. The samples were irradiated at 0 and 50 kGy in a cobalt-60 irradiator (Nordion International, Ottawa, Ontario, Canada). After irradiation, the analysis was performed and the remainder of the sample was immediately stored at 4 °C until its use in subsequent experiments.

2.2. Cell culture and animal treatment

RAW 264.7 macrophage cell was obtained from Korean Cell Line Bank (KCLB, Korea). Cells were maintained in RPMI-1640 including 10% of fetal bovine serum, penicillin–streptomycin of 100 units/ml at 5% in a CO_2 incubator and were seeded in 96-well tissue culture plates at a density of 5.0×10^5 cells/well and treated with β-glucan samples. After 24 h, supernatants were collected and stored until its analysis for cytokine assay of tumor necrosis factor-α (TNF-α) and nitric oxide (NO).

BALB/c mice were orally administrated with β-glucan samples for a period of 7 days (24 h interval) at a concentration of 50 mg/kg body weight. After 7 days, the mice were sacrificed and their spleens and Peyer's patch cells were isolated. Spleen and Peyer's patch cells were maintained same above medium in a CO_2 incubator and were seeded in 96-well tissue culture plates at a density of 1×10^6 and 5×10^4 cells/well, respectively, and treated with β-glucan. After 24 h, supernatants of spleen cells were collected and stored until its analysis for cytokine assay of interferon-γ (IFN-γ) and interleukin-2 (IL-2).

2.3. Analysis of cell proliferation, cytokine and nitric oxide

The proliferation of RAW 264.7 macrophage, spleen and Peyer's patch cells was detected by MTT (Sigma Chemical Co.) assay method. The levels of TNF-α, IFN-γ and IL-2 were measured using ELISA kits (BD Biosciences, CA, USA) according to the manufacturer's instructions. NO levels were determined by using the Griess Reagent, a common experimental measurement for nitrite, a metabolic byproduct of nitric oxide.

2.4. Statistical analysis

The results were analyzed by SPSS software for mean and standard deviation (Statistical Package for Social Sciences, 10.0, 2000). For cell proliferation, cytokine and NO release, SPSS ANOVA, LSD-test was used. A value of $p<0.05$ was considered statistically significant.

3. Results and discussion

3.1. Effect of β-glucan on RAW 264.7 macrophage cell

The effects of β-glucan on the proliferation of RAW 264.7 macrophage cells and the production of TNF-α and NO are shown in Fig. 1. The proliferation of macrophage cells was increased by the treatment of β-glucan in dose-dependent manner. Especially, irradiated β-glucan showed a greater proliferation of macrophage and release of TNF-α and NO when compared with non-irradiated β-glucan ($p<0.05$). TNF-α and NO are an excellent indicator of macrophage activation and play an important role in killing of tumor cells (Mantovani et al., 1992). Because β-glucan binds to iC3b-receptor, known as stimulates for phagocytosis and degranulation, with high affinity and prime the receptor for subsequent cytotoxic activation (Gordon et al., 1999).

Some researches have reported on correlations between the effectiveness of β-glucan and their molecular structure, size, branching frequency, structural modification, conformation and solubility (Chen and Seviour, 2007). Our previous study (Byun et al., 2008) reported that β-glucan was depolymerized by gamma irradiation depending upon the dose. The average molecular weight of non-irradiated β-glucan was found to be 178 kDa, while those of the irradiated at 10, 30 and 50 kGy were about 62, 32 and

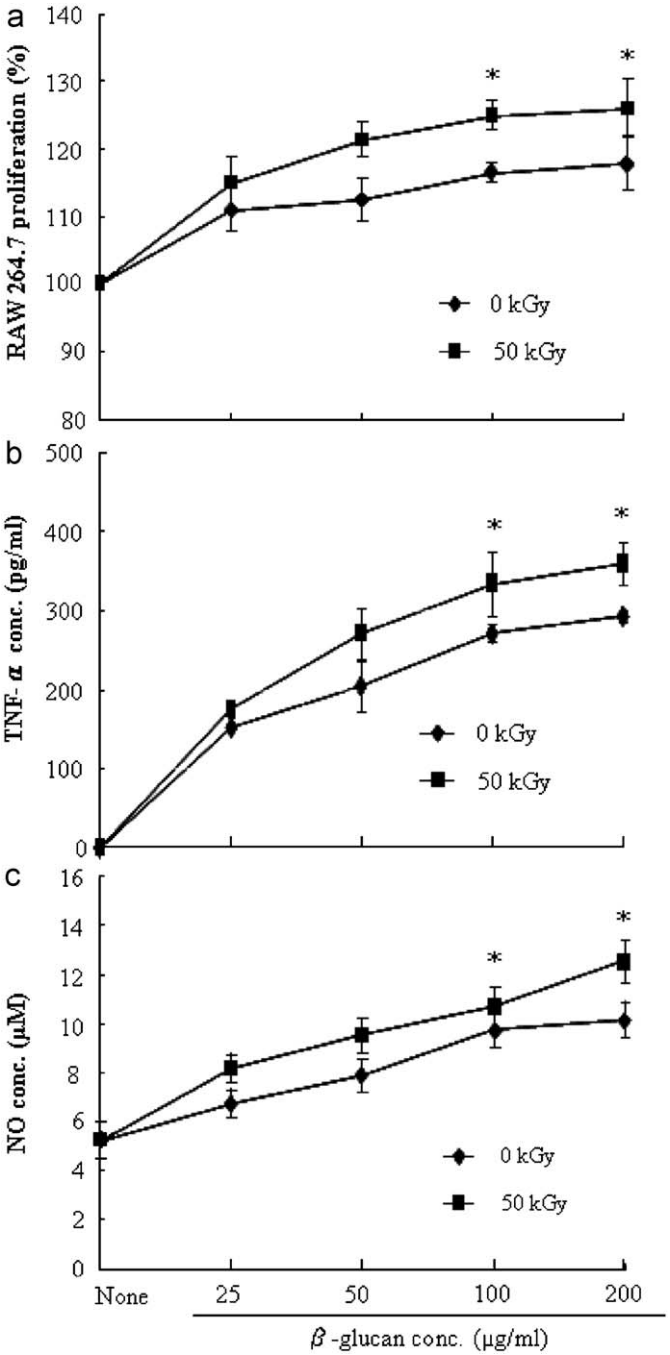

Fig. 1. Effects of non-irradiated (0 kGy) and irradiated β-glucan (50 kGy) on the proliferation of RAW 264.7 macrophage cell (a), and the production of TNF-α (b) and NO (c). Each experiment was run in triplicate. $^*p<0.05$, compared with 0 kGy.