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Antioxidant activities of *Ganoderma lucidum* polysaccharides and their role on DNA damage in mice induced by cobalt-60 gamma-irradiation

Wei Zhao, Xuejun Jiang, Wenwen Deng, Yanhao Lai, Mei Wu, Zunzhen Zhang *

Department of Environmental Health, School of Public Health, Sichuan University, Chengdu 610041, PR China

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ABSTRACT

In this study, the radio-protective effects of *Ganoderma lucidum* polysaccharides (GLP) were investigated in a mouse animal model exposed to ^{60}Co gamma-irradiation. Each of three batches of mice were divided into five groups (negative control, positive gamma irradiated control, and low, middle and high dosage GLP groups). Different batches of animals were used to evaluate the impact of GLP on peripheral white blood cell count, immune organ index; DNA damage, lipid peroxidation; micronuclei formation, and nucleated cell count in bone marrow induced by ^{60}Co gamma-irradiation. DNA strand-break and micronuclei frequency were significantly reduced and glutathione peroxidase activity and nucleated cell count in bone marrow were significantly increased by GLP treatment in a dose-dependent manner. GLP intervention also increased the activity of superoxide dismutase and decreased the level of malondialdehyde in middle and high GLP treatment groups. No adverse effects were observed on peripheral white blood cells and immune organ or body weight in either the control groups or GLP treated gamma exposed mice. These findings suggest that GLP possesses marked antioxidant capacity which plays an important role in the prevention of radiation damage in mice induced by ^{60}Co gamma-irradiation.

1. Introduction

Ionizing radiation (IR) exposure from both man-made and natural sources can impact human health in different ways and ionizing radiation causes a broad spectrum of acute, sub-chronic or chronic adverse effects including cell-killing, anti-proliferative, pro-inflammatory, pro-fibrotic and other biological effects (Ostrau et al., 2009). Genomic DNA is the molecule most susceptible to IR damage. Multiple studies have demonstrated that IR not only attacks genomic DNA, leading to single-strand breaks (SSB), double-strand breaks (DSB), base lesions, clustered damage, cross-links (DNA–DNA cross-links and DNA–protein cross-links) and oxidative base modification (Yokoya et al., 2008) but also reacts with free radicals to generate reactive oxygen species (ROS) resulting in initiation and enhancement of biomembrane lipid peroxidation (Kamat et al., 2000). Some anti-radiation drugs have been developed, but their widespread use is restricted due to their high cost, toxicity and regulatory limitations (Cui et al., 2010; Mujić et al., 2011; Devipriya et al., 2008). Over the past few years there has been an increased interest in the use of natural medicines and health foods to treat illness, including the use of natural products as natural anti-radiation substances (Hosseinimehr, 2007).

Ganoderma lucidum belongs to the family of *Ganodermataceae of Polyporales* and its fruiting body is generally called 'Lingzhi' in China. The use of *G. lucidum* as a traditional medicine can be dated back more than two millennia in China and is also well-known as a traditional medicine in other Pacific Rim countries such as Korea and Japan (Jia et al., 2009). Both *in vitro* and *in vivo* studies have shown that *G. lucidum* extract has a wide range of biological activities, including anticancer activity, antioxidation and immunestimulation (Paterson, 2006). Although *G. lucidum* contains various natural products, the *G. lucidum* polysaccharides (GLP) are generally considered to be the major source of its biological activities and therapeutic uses (Gao et al., 2004). Data obtained from both tissue culture and animal studies have shown that GLP has protective effects against oxidative damage caused by physical and/or chemical agents. However, these studies have limitations: (i) Most of the studies were carried out in non-biological systems or with *in vitro* cell exposures (Liu et al., 2010; Pillai et al., 2010) and those studies using animal models have initiated oxidative damage by

Abbreviations: ^{60}Co, cobalt-60; IR, ionizing radiation; GLP, *Ganoderma lucidum* polysaccharides; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; WBC, white blood cell; OTM, olive tail moment; PCE, polychromatic erythrocytes; MPCE, micronuclei of polychromatic erythrocytes; MN, micronuclei; ROS, reactive oxygen species; NMPA, normal melting point agarose; LMPA, low melting point agarose.

* Corresponding author. Address: Department of Environmental Health, Sichuan University, West China School of Public Health, No. 16, Section 3, Renmin Nanlu, Chengdu 610041, PR China. Tel.: +86 28 85501298; fax: +86 28 85501295.
E-mail addresses: zhangzz@scu.edu.cn, zhangzunzhen@163.com (Z. Zhang).

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adriamycin and ischemic reperfusion instead of gamma-irradiation (Pan et al., 1998; Shi et al., 2010); (ii) Previous studies that have induced oxidative damage with gamma-irradiation, have used dosages much higher than those humans are likely to be exposed under normal or even occupational circumstances. Thus the study results were inappropriate for application to radiation protection (Li et al., 2007); (iii) The parameters evaluated primarily focused on enzymatic antioxidants and lipid peroxidation products, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and malonaldehyde (MDA) (Li et al., 2007; Shi et al., 2010), with limited emphasis on DNA oxidative damage and micronuclei induction; (iv) Few studies have evaluated the impact on peripheral white blood cell count and nucleated cell count in bone marrow; and, (v) Previous studies have focused on the biochemical effects of the fruiting body or methanolic or phenolic extracts of *G. lucidum* instead of GLP from sporoderm-broken spore (Li et al., 2007; Lakshmi et al., 2006; Liu et al., 2010; Shi et al., 2010).

In this study, we investigated the radio-protective potential of polysaccharides from sporoderm-broken spores of *G. lucidum* (GLP) on oxidative damage induced by gamma-irradiation in mice. Specific studies focused on radiation induced DNA strand-breaks, micronuclei formation, white blood cell and bone marrow nucleated cell counts, and lipid peroxidation.

2. Materials and methods

2.1. Material and animal

G. lucidum sporoderm-broken spore powder was provided by Chuanda Jinzhong Science and Technology Co. Ltd (Sichuan, China; batch number: 100901) and more than 99% of spores were sporoderm-broken. The sporoderm-broken spore powder contained 2% GLP by weight as determined by high performance liquid chromatography (HPLC) by the Department of Sanitary Technology, School of Public Health, Sichuan University. For animal exposures, *G.* lucidum sporoderm-broken spore powder was dissolved in distilled water at concentrations of 3.33, 6.67 and 10.00 mg/ml and mice were intragastric administration 2 ml/100 g of these aqueous solutions which were prepared just before use.

One hundred and fifty specific pathogen free (SPF) ICR female mice (six to eight weeks old, weighing 18–22 g) were ordered from Chengdu Dashuo Biological Science Co. Ltd (certification number: SCXK 2008-24). Animals were housed in individually ventilated cages (IVC) containing sterile wood chips as bedding in an air-conditioned room under standard laboratory conditions (temperature: 22 °C–23 °C, relative humidity: 61–65%, 12 h light and 12 h dark) at the IVC animal experimental center, School of Public Health, Sichuan University. Animals had access to tap water and standard commercial mouse chow (pellet form, laboratory animal center of Sichuan University, Chengdu, China) ad libitum and were quarantined five days prior to dosing. The protocol followed the guidelines approved by local Ethics Committee. All experimental procedures were conducted in accordance with the Guide to the Care and Use of Laboratory Animals.

2.2. Experimental design

The experimental design followed the Technical Standard for Testing and Assessment of Health Food (Ministry of Health, China, 2003) (Table 1). One hundred and fifty SPF mice were randomized into three batches (A, B and C) equally. Mice of batch A were used to evaluate the effects of GLP on white blood cell counts, spleen and thymus after gamma-irradiation. Mice of batch B were used to evaluate the protective effects of GLP against lipid peroxidation and DNA damage caused by gamma-irradiation. Mice of batch C were used to assess the effects of GLP on radiation induced chromosomal aberration and nucleated cell levels. The body weight of each mouse was recorded as a reference variable throughout the process of this study.

Each batch was randomly divided into five groups: negative control, positive control, low, middle and high dosage GLP groups. Each group contained ten mice. Mice were exposed daily by intragastric administration to aqueous solutions of *G. lucidum* spore powder at dosing levels of 0.67, 1.33 and 2.00 g/kg. These dosing levels were equivalent to 13.4, 26.6 and 40.0 mg/kg of GLP, respectively. The mice of control groups were given distilled water daily on the base of equal-volume intragastric administration. All mice, except those from the negative control group, were exposed to ^{60}Co gamma-irradiation as described below.

2.3. Cobalt-60 gamma-irradiation

GWXJ80 ^{60}Co gamma radiotherapy equipment (GWXJ80 ^{60}Co gamma telecobalt unit, nuclear power institute of China) of No.363 hospital (Chengdu, China) was used for irradiation. Ten individual mice from each group were placed in a well-ventilated plexiglass box with dimension of 20.0 cm × 20.0 cm × 3.0 cm and exposed to whole body gamma-irradiation to deliver the required dosage at the dose rate of 34.33 cGy/min (Table 1). In this way, the mice were confined to a small space without moving around so as to ensure the accuracy of gamma-irradiation dosage.

2.4. White blood cell count and immune organ index

Twenty microlitres of peripheral blood from the mouse tail was collected 1 week prior to, 3 days and 2 weeks after irradiation to evaluate the effects of GLP on peripheral WBC level. The peripheral blood was added to 0.18 ml 1% HCl after which a blood counting chamber was used to count the WBC (N). WBC levels were calculated using the following formula: WBC (10^9/L) = (N/4) × dilution fold × 10^7. To estimate the effects of GLP on immune system, all mice were sacrificed immediately after the last blood-sampling and the spleen and thymus were removed and weighed for calculation of the immune organ index expressed as grams (spleen and thymus)/kg.

2.5. Comet assay and SOD, GPx, MDA measurement

Approximately 0.2 g of the liver from each mouse was minced and washed with precooled phosphate buffered saline (PBS) to remove the red blood cells. The minced livers were forced through a wire mesh screen to collect single-cell suspensions. The cell viability was checked by trypan-blue dye-exclusion technique and single cell gel electrophoresis was carried out based on methods from our laboratory (Liu et al., 2011). Briefly, the single-cell suspensions were mixed with 0.65% molten LMPA (Amresco Inc. Solon, Ohio, USA) and spread on slides precoated with 0.80% NMPA (Amresco Inc. Solon, Ohio, USA). The slides were covered with coverslips and allowed to solidify for 10 min at 4 °C; afterwhich the coverslips were removed and the slides were placed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 1% Triton X-100, 10% DMSO; the last two components were freshly added) for 1 h at 4 °C. After lysis, the slides were exposed to freshly prepared electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH > 13) for 30 min to allow unwinding of DNA afterwhich the slides were electrophoresed for 30 min in an electric field of 0.75 V/cm (300 mA). After electrophoresis, the microgels were neutralized with distilled water and stained with 45 μl of 20 μg/ml ethidium bromide (Sigma–Aldrich Inc, St. Louis, MO,USA) just prior to analysis. The comet cells were analyzed at 200× magnification in a fluorescence microscope (Leica, Germany) attached to a digital camera (Nikon, Japan) and connected to a computer. Comet rate and OTM value were chosen to reflect the DNA damage extent. OTM value incorporates both the smallest detectable DNA migration reflected by the length of the comet tail and the number of broken pieces of DNA represented by the staining intensity of DNA in the tail. Two hundred randomly selected cells were scored from each slide and the rate of comet cells was calculated; OTM of 30 randomly selected comet cells was detected with the software 'Comet Assay Software Project' (CASP).

Table 1
Experimental design.

Batch	Group[a]	Radiation dose(Gy)[b]	Radiation date	Execution date	Experiments
A	5	3	16 days after the beginning of dose	2 weeks after the radiation[c]	White blood cell count and immune organ index
B	5	6	4 weeks after the beginning of dose	1 week after the radiation	Comet assay and SOD, GPx, MDA measurement
C	5	3	4 weeks after the beginning of dose	3 days after the radiation	Micronuclei assay and bone marrow nucleated cell count

[a] (150 SPF mice were randomized into three batches and each of three batches of mice were divided into 5 groups, i.e. negative control, positive control, and low, middle and high dosage GLP groups.).
[b] (Mice from negative control were not exposed to gamma-irradiation).
[c] (Twenty μl of peripheral blood from the mouse tail of batch A were collected 1 week prior to, 3 days and 2 weeks after the radiation).