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Original paper

Radioprotective effects of Dragon's blood and its extract against gamma irradiation in mouse bone marrow cells

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ABSTRACT

Purpose: The radioprotective effects of Dragon's blood (DB) and its extracts (DBE) were investigated using the chromosomal aberrant test, micronucleus and oxidative stress assay for anti-clastogenic and anti-oxidative activity.
Materials and methods: Adult BALB/C mice were exposed to the whole body irradiation with 4 Gy ^{60}Co γ-rays. DB and DBE were administered orally once a day from 5 days prior to irradiation treatment to 1 day after irradiation. The mice were sacrificed on 24 h after irradiation. The cells of bone marrow were measured by counting different types of chromosomal aberrations and the frequency of micronuclei. Oxidative stress response was carried out by analysis of serum from blood.
Results: DB and DBE significantly decreased the number of bone marrow cells with chromosome aberrations after irradiation with respect to irradiated alone group. The administration of DB and DBE also significantly reduced the frequencies of micronucleated polychromatic erythrocytes (MPCE) and micronucleated normochromatic erythrocytes (MNCE). In addition, DB and DBE markedly increased the activity of antioxidant enzymes and the level of antioxidant molecular. Malondialdehyde (MDA) and nitric oxide (NO) levels in serum were significantly reduced by DB and DBE treatment.
Conclusions: Our data suggested that DB and DBE have potential radioprotective properties in mouse bone marrow after ^{60}Co γ-ray exposure, which support their candidature as a potential radioprotective agent.

Introduction

Ionizing radiation interacts with the living body to generate free radicals or reactive oxygen species (ROS), which further lead to deleterious effects on living and normal cells by attacking various cellular components including DNA, proteins and membrane lipids [1]. Radiation-induced DNA lesions, such as single strand, double strand breaks, chromosomal aberration and various mutations are associated with an increased risk of numerous genetic diseases [2,3]. High dose radiation can induce the accumulation of cellular damage, leading to dysfunction and diseases like neurological disorders and cancer, or even death in mammals [4]. Therefore, effectively scavenging the ROS and inhibiting the DNA damage are thought to be potential approaches for providing protection against radiation injury, A large number of natural and synthetic compounds have been evaluated for this purpose, for example, tocotrienols, amifostine, CBLB502, ON1210.Na etc. [5–7].

Dragon's blood (DB) is a bright red resin obtained from *Dracaena cochinchinensis* (Lour.) S.C.Chen (China). As a traditional Chinese medicine, it has been widely used for the treatment of wound, blood stasis, allergic dermatitis, inflammation etc. [8]. Phytochemical studies on DB have identified the presence of flavanoids, homoisoflavanoids, terpenoids, steroids and chalcones [9]. Being rich in phenolic compounds the resin has antioxidative activity and chemoprotective potential [10]. We previously found that DB has radioprotective properties, including reduction of oxidative stress, inflammatory cytokines and neuronal apoptosis after whole brain irradiation of rats with either heavy ions or γ-rays [11]. Therefore, it is essential to have more definite information on anti-radiation

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potential of this resin. In this study, we investigate the radioprotective effects of Dragon's blood and its extracts (DBE) against radiation induced oxidative stress alterations and DNA damage and the induction of micronucleus of bone marrow in mouse exposed to γ-rays.

Materials and methods

Animals

Male BALB/c mice weighing 25 ± 3 g were purchased from Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). Animal experiment protocols were approved by Beijing Institute of Technology Animal Care and Use committee. The study complied with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Mice were housed and given standard mouse pellets and water ad libitum. All animals were maintained under 12 h light/dark cycle at a temperature approximately (23 ± 2) °C.

Drugs and treatment

DB (No. 20061120) was provided by Beijing BIT&GY Pharmaceutical R&D Co., Ltd. DB was dissolved in 83% ethanol (Beijing chemical works) until completely dissolved at room temperature, and then diluted with 50% ethanol and kept for 24 h at room temperature to separate a clear supernatant and precipitate phase. The precipitate phase was dissolved in 83% ethanol one more time and repeated the whole previous process. The primary clear supernatant and the secondary clear supernatant were combined together for the experiment proposes. The 50% ethanol extract (DBE, 80 g) was prepared by concentration and desiccation. Phenolic compounds were identified by Beijing BIT&GY Pharmaceutical R&D Co., Ltd. to be the main active components of DBE (Fig. 1).

The mice were divided into 4 groups as follows, each group contains 10 mice:

1) Control group, in which mice are orally administrated with solvent control [0.5% sodium carboxymethylcellulose, 0.5% CMC-Na, 0.5 g/kg body weight (b.wt.)] and treated with sham irradiation;
2) Irradiation-alone group, in which mice are orally administrated with solvent control (0.5 g/kg b.wt.) and treated with 4 Gy γ-rays;
3) DB-treated group, in which mice are orally administrated with DB (375 mg/kg b.wt.) and treated with 4 Gy γ-rays;
4) DBE-treated group, in which mice are orally administrated with DBE (300 mg/kg b.wt.) and treated with 4 Gy γ-rays.

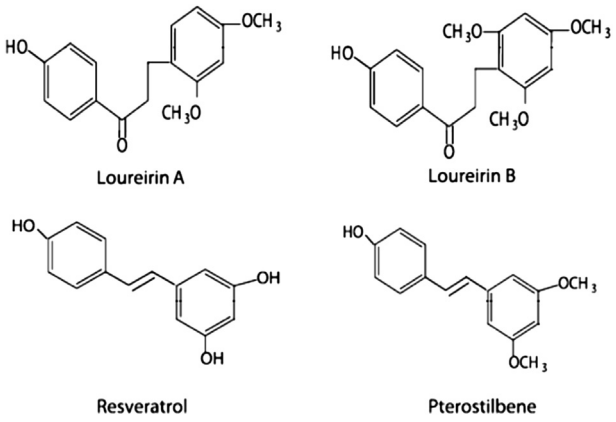

Figure 1. Main chemical components of Dragon's blood extracts.
Dragon's blood (No. 20061120) and Dragon's blood extracts were provided by Beijing BIT&GY Pharmaceutical R&D Co., Ltd.

All groups of mice are orally administrated with solutions respectively, as we describe above, from 5 day prior to irradiation to 1d after irradiation. On the day of irradiation, the animals were exposed to 4 Gy γ-rays at 1 h after the oral administrations. All of the animals were sacrificed on 24 h after irradiation.

Irradiation

Whole-body irradiation was performed with a cobalt-60 γ-radiation source, which was provided by the Military Medical Science Academy of the PLA, Beijing, China. Mice were placed in ventilated Plexiglas cages and irradiated in groups of five mice, simultaneously. The source-to-skin distance was 400 cm with a dose rate of 169.89 cGy min^{-1} at room temperature (23 ± 2 °C). The mice were irradiated with a total dose of 4 Gy γ-rays.

Chromosomal aberrations examination

The mice were given a single intraperitoneal (i.p.) injection of 0.04% colchicine (4 μg/kg b.wt., Solarbio) at 22 h after irradiation and then sacrificed 2 h later by cervical dislocation. Both femurs of each animal were dissected and cleaned from the muscles. Metaphase plates were prepared by the air-drying method [12]. The bone marrow from femurs was flushed into a centrifuge tube using 0.9% physiological saline (Beijing chemical works). The cells were gently pipetted up and down several times. The cells were centrifuged at 190× *g* for 8 min at 4 °C, the supernatant was discarded and bone marrow cells were harvested. The cell pellet was resuspended in 0.565% KCl (Beijing chemical works) and treated hypotonically for 20 min, pre-fixed in stationary liquid (3:1: methanol:acetic acid, Beijing chemical works), and then centrifuged at 190× *g* for 8 min at 4 °C. This process has to be repeated three times. The smears were spread on clean, dry slides, and were stained with 4% Giemsa (Sigma). The slides were observed under microscope (Olympus IX71) to count different types of chromosomal aberrations like chromatid breaks, chromosome breaks, fragments, rings and dicentrics as well as cells showing polyploidy and severe damage [13,14]. For each mouse, total of 100 metaphase cells were counted. The number of aberrant metaphases is represented as percent aberrant metaphases and the different aberrations are expressed as aberrations per 100 cells.

Micronucleus assay

The method for bone marrow micronucleus test was used as described by Schmid with some modifications to evaluate the chromosomal damage in experimental animals [15,16]. The bone marrow cell pellet was suspended in a few drops of fetal calf serum (FCS, Gibco). After 24 h air-drying and fixed in absolute methanol for 15 min at room temperature, the smears were stained with May-Grunwald/Giemsa (Sigma) to produce the differential staining of polychromatic erythrocytes (PCE) as reddish-blue cells due to their high-RNA content and normochromatic erythrocytes (NCE) as orange cells. The nuclear material of PCE and NCE was also stained with dark purple. The slides were observed under microscope. For each mouse, a total of 2000 erythrocytes were counted and the number of PCEs and NCEs and the frequency of micronucleated normochromatic erythrocytes (MNCE) and micronucleated polychromatic erythrocytes (MPCE) were analyzed. The ratio of MPCE/MNCE was evaluated separately by counting the number of MPCE