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Effects of Ligustrazine on DNA damage and apoptosis induced by irradiation

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ABSTRACT

Ligustrazine has been used to treat heart and blood vessel disease in China. In the present study, we investigated the potential action of Ligustrazine as a component of *chuanxiong* (a Chinese herb) in scavenging hydroxyl radical and superoxide radical as indicated in the ESR spin-trapping measurement. Treatment of Ligustrazine in mice decreased mortality after whole body γ-irradiation. The anti-radiation action of Ligustrazine was studied by measuring DNA damage (Comet assay and γ-H2AX formation) and apoptosis induced by irradiation. It was triggered by altering the level of DNA-PKcs protein, a critical component of DNA double-strand break (DSB) repair pathways in mice after irradiation. Consistently, the phosphorylation of Akt protein, a mediator of survival signaling, was concurrently increased by Ligustrazine treatment. Additionally, the cytokines along with the phosphorylation of the p38 protein which is activated by a variety of environmental stresses and inflammatory cytokines decreased in the Ligustrazine-treated group as compared to irradiation group. Our results suggest that Ligustrazine has radioprotective effect through its capabilities as a powerful antioxidant, in reducing reactive oxygen species (ROS) level induced by irradiation, minimizing DNA damage and apoptosis, and activating survival signal Akt pathways. This study will be of value in the development of novel radioprotective compounds.

1. Introduction

Ionizing radiation (IR) has been used in medicine (specifically radiotherapy and radiodiagnosis), industry, and research laboratories for several decades. However, radiation exposure leads to hydrolysis of water and the generation of reactive oxygen species (ROS) (Papa and Skulachev, 1997), which can damage macromolecules, including DNA, cell membranes and enzymes and gives rise to genomic instability leading to mutagenesis, carcinogenesis, and cell death (Huang et al., 2003; Citrin et al., 2010). Exposure to γ-radiation at sub-lethal doses induces a variety of cellular and sub-cellular damage in many living organisms (Garrison and Uyeki, 1988). Therefore, protection of biological systems against radiation damage is of paramount importance.

DNA double-strand break (DSB) is the critical lesion induced by ionizing radiation, and it is believed to be the key trigger leading to a series of cellular consequences induced by radiation, e.g. cell death, gene mutation and chromosome aberrations (Zheng et al., 2008; Mah et al., 2010). There are two major intrinsic factors deciding the extent of DNA damage in

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the irradiated cells, the activity of antioxidant systems and the capacity of DNA repair. There are two distinct but complementary mechanisms for DNA DSB repair, non-homologous end joining (NHEJ) and homologous recombination (HR) – both of which are complicated cascades involving various repair proteins (Hefferin and Tomkinson, 2005; Sonoda et al., 2006; Zheng et al., 2008). DNA dependent protein kinase (DNA-PK) complex, consisting of three subunits: the catalytic subunit DNA-PKcs, and the regulating subunits Ku70 and Ku80, has been identified to play an important role in NHEJ pathways (Smith and Jackson, 1999; Khanna and Jackson, 2001; Chan et al., 2002).

It is still an uphill task to locate an ideal antioxidant radioprotective agent of antioxidant and to minimize DNA damage. Although efforts to find suitable radiation countermeasures have continued for more than six decades and various radioprotective strategies have been explored, including thiols, growth factors, and cytokines (Weiss and Landauer, 2000; Vijayalaxmi et al., 2004), no safe and effective radioprotectants were addressed. Numerous candidate radiation countermeasures have been identified and investigated, but there was often a parallel increase in the deleterious side effects, including relatively high toxicity and unfavorable routes of administration, which delay their application and efficacy (Maisin, 1998). One of the most potent protective compounds, the phosphorothioate amifostine, was approved by the FDA for the prevention of radiation-induced xerostomia (Weiss and Landauer, 2009); another candidate is a polypeptide drug derived from flagellin secreted by *Salmonella typhimurium* (Burdelya et al., 2008), designated as CBLB502, reduced both hematopoietic (10 Gy) and GI damage (13 Gy) in mice when administered 30–60 min before irradiation (Burdelya et al., 2008). These agents, however, no exception have many adverse effects making them unacceptable for prophylactic use in radiation emergencies in the nonclinical population. Therefore, the search for new, less toxic radioprotectors is crucial to develop improved strategies for protecting normal cells from radiation-induced damage.

We have shown that the application of Ligustrazine (tetramethy pyrazine), a component contained in Chuanxiong (Ligusticum chuanxiong Hort, one of the ABCRBS herbs), exhibits protective effects on burn-induced organ injury (Zheng et al., 2005, 2006; Gao et al., 2007). Ligustrazine has impressive effects in scavenging cytotoxic oxygen free radicals, promoting blood flow, antiplatelet aggregation (Feng et al., 2004a,b). Previously, we demonstrated that Ligustrazine alleviated burn-mediated hepatic dysfunction by its inhibitory action on the activation of NF-kB following burn trauma. However, there are few reports regarding the effect of Ligustrazine on radioprotective and DNA damage. Therefore, the aim of our present study is to investigate the radioprotective effects of Ligustrazine on irradiation induced oxidative damage.

In the present study, we investigated detailed effects of Ligustrazine to protect mice from injuries induced by irradiation, based on the previous observation. We also characterized its mechanisms of action, through assaying antioxidant properties, DNA damage and repair, and the changes of inflammatory cytokines. The expression and/or phosphorylation changes were further investigated for DNA-PKcs, the critical components involved in the NHEJ pathway of DNA DSB repair, as well as Ser/Thr kinase Akt (protein kinase B) which is considered to be a mediator of cell survival signal pathways.

2. Materials and methods

2.1. Animals

All animal procedures complied with the Animal Welfare and Ethics Committee of Anhui Medical University Guidelines for the Care and followed their guidelines for animal experimentation. 8–10-Week-old male C57BL/6 mice purchased from the Laboratory Animal Center, Anhui Medical University were used for the study. All mice were randomly assigned to experimental groups. Six mice were housed in filter-topped polycarbonate cages in a temperature controlled room with alternating 12-h light:12-h dark cycles, and were allowed 1 week to acclimate to their surroundings. Mice were fed a standard animal diet; food and tap water would be available at will throughout the experimental protocol.

2.2. Irradiation

Mice were given selected doses of whole-body ^{60}Co γ-photon radiation delivered at a dose rate of 226 cGy per minute. Mice treated as shams (sham group) were placed into the irradiator for matched amounts of time but without exposure to the source and were not injected with Ligustrazine. Ligustrazine was administered intravenously at doses of 20, 40 and 80 mg/kg in the mice prior to irradiation (Ligustrazine group), while mice in the irradiation group (IR group) were given identical saline solution in the same manner. After 9.5 Gy irradiation, survival for 30 days was measured.

2.3. ESR spin-trapping measurements for free radicals

The reaction of xanthine and xanthine oxidase was used as a source of superoxide radical. The reaction mixture consisted of dimethyl pyrroline-*N*-oxide (DMPO, 50 mM), xanthine (20 μM) and xanthine oxidase (0.01 U/mL) in air-saturated phosphate-buffered solution in the absence or presence of 10 μM Ligustrazine. The reaction was started by adding the xanthine oxidase stock, which was prepared in 10 mM sodium/potassium phosphate-buffered solution (pH 7.4). The Fe^{2+}/H_2O_2 reaction system was used as the source of hydroxyl radical. Individual samples were placed in the ESR cavity, and ESR spectra were recorded using the ESP300 ESR spectrometer (Bruker, Germany).

2.4. Determination of the effect of Ligustrazine on γ-radiation induced DNA damage by comet assay

Animals received a single exposure of whole body ^{60}Co γ-radiation (6 Gy/animal). At the indicated time after irradiation, animals were killed by cervical dislocation. Bone marrow cells from each femur were collected. The bone marrow cells from irradiated and Ligustrazine-treated mice were flushed into PBS and then were collected and mixed with low melting point (LMP) agarose at 37 °C. This mixture was placed on the top of the previous layer of 0.5% normal melting point (NMP) agarose