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Research article

Effects of Korean Red Ginseng extract on busulfan-induced dysfunction of the male reproductive system

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ABSTRACT

Background: Anticancer agents induce a variety of adverse effects when administered to cancer patients. Busulfan is a known antileukemia agent. When administered for treatment of leukemia in young patients, busulfan could cause damage to the male reproductive system as one of its adverse effects, resulting in sterility.
Methods: We investigated the effects of Korean Red Ginseng extract (KRGE) on busulfan-induced damage and/or dysfunction of the male reproductive system.
Results: We found that administration of busulfan to mice: decreased testis weight; caused testicular histological damage; reduced the total number of sperm, sperm motility, serum testosterone concentration; and eventually, litter size. Preadministration of KRGE partially attenuated various busulfan-induced damages to the male reproductive system. These results indicate that KRGE has a protective effect against busulfan-induced damage to the male reproduction system.
Conclusion: The present study shows a possibility that KRGE could be applied as a useful agent to prevent or protect the male reproductive system from the adverse side effects induced by administration of anticancer agents such as busulfan.

1. Introduction

Ginseng, the root of *Panax ginseng* Meyer, has been used as a representative tonic for over 2,000 yr in Far East countries, and currently ginseng is one of the most famous and precious herbal medicines consumed around the world [1]. Although ginseng exhibits diverse pharmacological actions *in vitro* and *in vivo*, the detailed mechanisms of its various efficacies are still elusive [2]. Ginseng might contain at least two components that are responsible for its diverse medicinal effects. Ginseng saponins (or ginsenosides) are one of the main active ingredients of ginseng. Ginseng saponins are glycoside saponins and derivatives of triterpenoid dammarane, which consists of 30 carbon atoms. Ginsenosides exhibit diverse pharmacological effects involving multiple

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mechanisms [2]. In addition, a recent study showed that ginseng also contains the G protein-coupled lysophosphatidic acid (LPA) ligand, *gintonin*. The primary action of gintonin is to induce $[Ca^{2+}]_i$ transient through the activation of LPA receptors with high affinity [3]. Gintonin also regulates Ca^{2+}-dependent ion channels and receptors and further exhibits anti-Alzheimer's disease and anti-metastatic effects *in vivo* [4,5].

Busulfan is a cell-cycle nonspecific alkylating antineoplastic agent that belongs to the class of alkyl sulfonates. As an alkylating agent, busulfan forms DNA–DNA intrastrand crosslinks between the DNA bases guanine and adenine and between guanine and guanine [6]. This DNA crosslinking by busulfan prevents DNA replication, and the cellular machinery cannot repair DNA crosslinks; thus, the cancer cells undergo apoptosis [7]. Busulfan is used for the chemotherapeutic treatment of chronic myeloid leukemia, as it is a low-cost drug. However, following treatment, busulfan exhibits adverse effects in various organs, including the reproductive system. For example, busulfan treatment induces azoospermia and testicular atrophy in young male patients, resulting in sterility in certain cases.

In a previous report, it was shown that Korean Red Ginseng extract (KRGE) has *in vitro* and *in vivo* anticancer activity against various cancers [8]. However, little is known or not KRGE also attenuates the adverse effects induced by anticancer agents. Kim et al [9,10] showed that oral administration of KRGE attenuates cisplatin-induced nausea and vomiting in experimental animals. Thus, these results indicate that KRGE might also have an attenuating effect on the adverse effects induced by anticancer agents, however, the possibility that KRGE could also be applied to attenuate adverse effects that are induced by anticancer agents other than cisplatin has not been reported.

2. Materials and methods

2.1. Materials

Korean Red Ginseng (KRGE) is manufactured by Korea Ginseng Corporation (Seoul, Korea) from the roots of 6-yr-old red ginseng plants (*P. ginseng* Meyer) harvested in the Republic of Korea. Korean Red Ginseng was prepared by steaming fresh ginseng at 90–100°C for 3 h and then drying at 50–80°C. KRGE was prepared from Korean Red Ginseng, which was extracted at 85–90°C for 8 h by circulating hot water three times. The water content of the pooled extract was 36% of the total weight. KRGE was analyzed using high-performance liquid chromatography. KRGE contained the major ginsenosides, including Rb1, 7.44 mg/g; Rb2, 2.59 mg/g; Rc, 3.04 mg/g; Rd, 0.91 mg/g; Re, 1.86 mg/g; Rf, 1.24 mg/g; Rg1, 1.79 mg/g; Rg2, 1.24 mg/g; Rg3, 1.39 mg/g; Rh1, 1.01 mg/g, and other minor ginsenosides. All other analytical reagents were obtained from Sigma (St. Louis, MO, USA).

2.2. Animals

Imprinting Control Region (ICR) male mice (age, 6 wk; weight, 28–32 g) were used in experiments evaluating the size and weight of the testis. Thirty male mice were divided into six equal groups (Fig. 1). Each group received saline or red ginseng (300 mg/kg, oral administration); the busulfan control group received saline and busulfan (40 mg/kg, intraperitoneal injection); all the other groups received KRGE orally (100 mg/kg, 200 mg/kg, or 300 mg/kg); in addition, these three groups also received busulfan intraperitoneally (40 mg/kg) [11]. All groups received KRGE or saline orally for 5 wk. Mice were treated with a single intraperitoneal injection of busulfan 1 wk prior to performing the study. All experiments were conducted in accordance with the National Institutes of Health Guide of Laboratory Animals. The study protocol was approved by the Institutional Animal Care and Use Committee of the Konkuk University (Seoul, Korea).

2.3. Body weight, testis weight, and sperm livability

Mice in all groups were killed after 5 wk. The testis was weighed at the time of killing without removing the tunica. The epididymis was clamped in each mouse to determine sperm count. The cauda was dissected and transferred to Dulbecco's modified Eagle medium

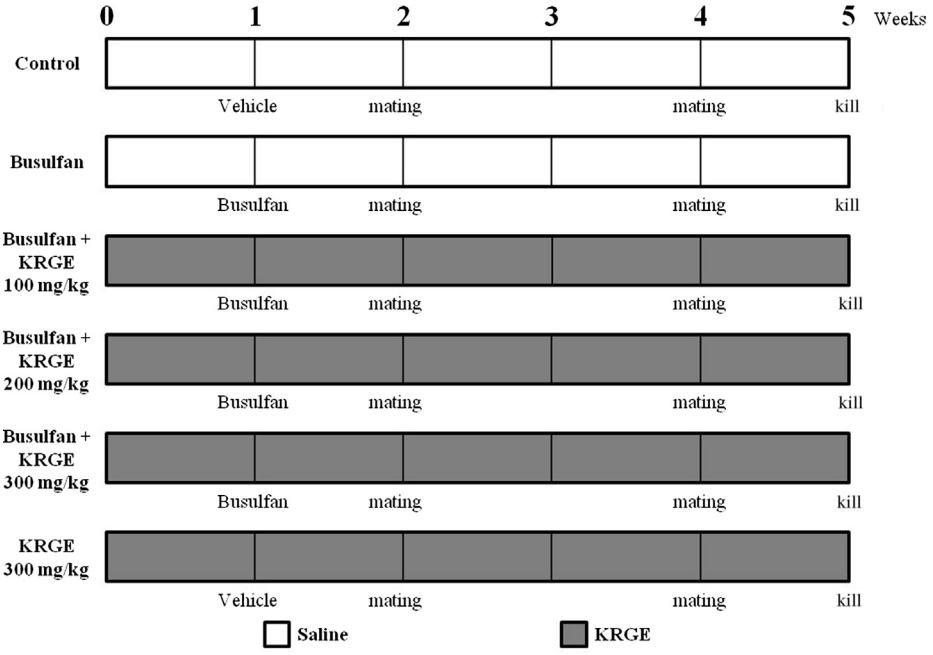

Fig. 1. Experimental protocols. Busulfan was injected intraperitoneally at a single dose of 40 mg/kg. Each group was administered a single gavage dose of Korean Red Ginseng extract (KRGE; 100 mg/kg, 200 mg/kg, or 300 mg/kg) or saline (control) for 5 wk.