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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

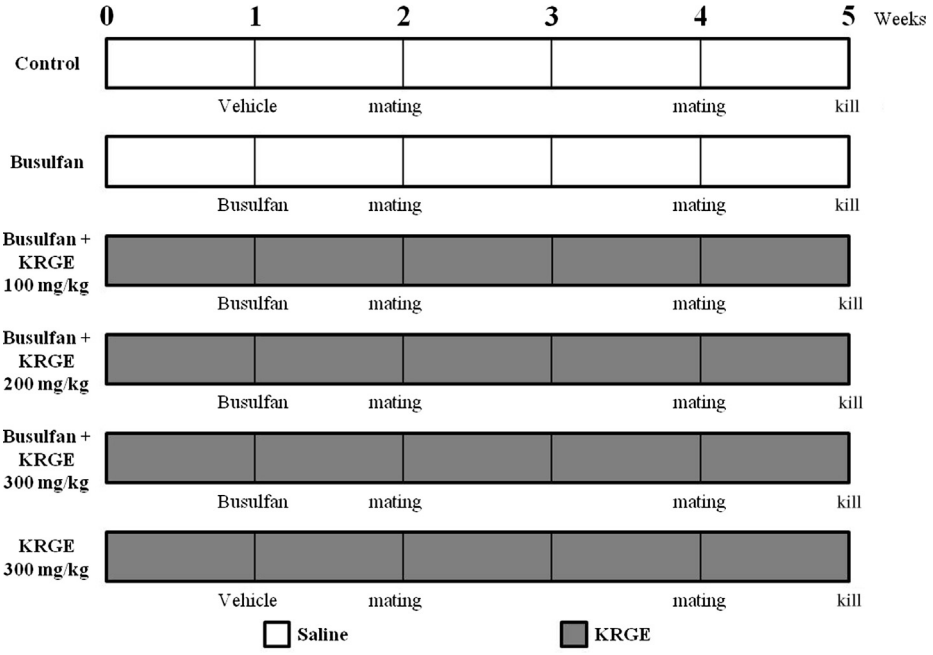

Fig. 1. Experimental protocols. Busulfan was injected intraperitoneally at a single dose of 40 mg/kg. Each group was administered a single gavage dose of Korean Red Ginseng extract (KRGE; 100 mg/kg, 200 mg/kg, or 300 mg/kg) or saline (control) for 5 wk.

(DMEM) supplemented with 0.5% bovine serum albumin (BSA) at 37°C [12]. Sperm remaining in the epididymis were washed and gathered in DMEM. Cauda sperm counts were measured using a Burker hemocytometer (Neubauer, Darmstadt, Germany) [12]. Sperm livability was estimated using trypan blue stain.

2.4. Sperm analysis

Epididymal sperm count and motility were evaluated as described by Connolly et al [13], with some modifications. To obtain the sperm count, the entire epididymis from the mouse was minced in M199 media (CureBio, Seoul, Korea) containing 0.5% BSA and incubated for 5 min at 37°C. The sperm concentration was determined by manual evaluation using a hemocytometer (Neubauer). For assessment of sperm motility, sperm were recovered from the excised cauda epididymis and allowed to capacitate for 5 min in M199 media containing 0.5% BSA at 37°C. Sperm were scored as motile if any movement was detected. The total number of sperm and the number of sperm that were motile were determined [14]. The percentage of motile spermatozoa in each treatment group was calculated by the Integrated Semen Analysis System.

2.5. Histological analysis

The testes of busulfan-treated mice were fixed in Bouin solution and embedded in paraffin. Sections of 5-μm thickness were stained with hematoxylin and eosin and observed under a light microscope [12]. For measurement of seminiferous tubule diameter, images of the testis sections were acquired using a 10× or 4× objective lens and 50 tubules/section were randomly selected and measured using the *line measure* option in the NIS-Elements software (Nikon, Tokyo, Japan) [15].

2.6. Reproductive potential of busulfan-treated mice

One male was mated with two ICR female mice for 5 d (male, $n = 5$; female, $n = 10$), so that each male had two chances for successful mating, resulting in pregnancy. After 20 d, we checked the litter size of each busulfan-treated group.

2.7. Testosterone analysis

Radioimmunoassay (RIA) for serum testosterone was carried out using Coat-A-Count total testosterone kit according to the

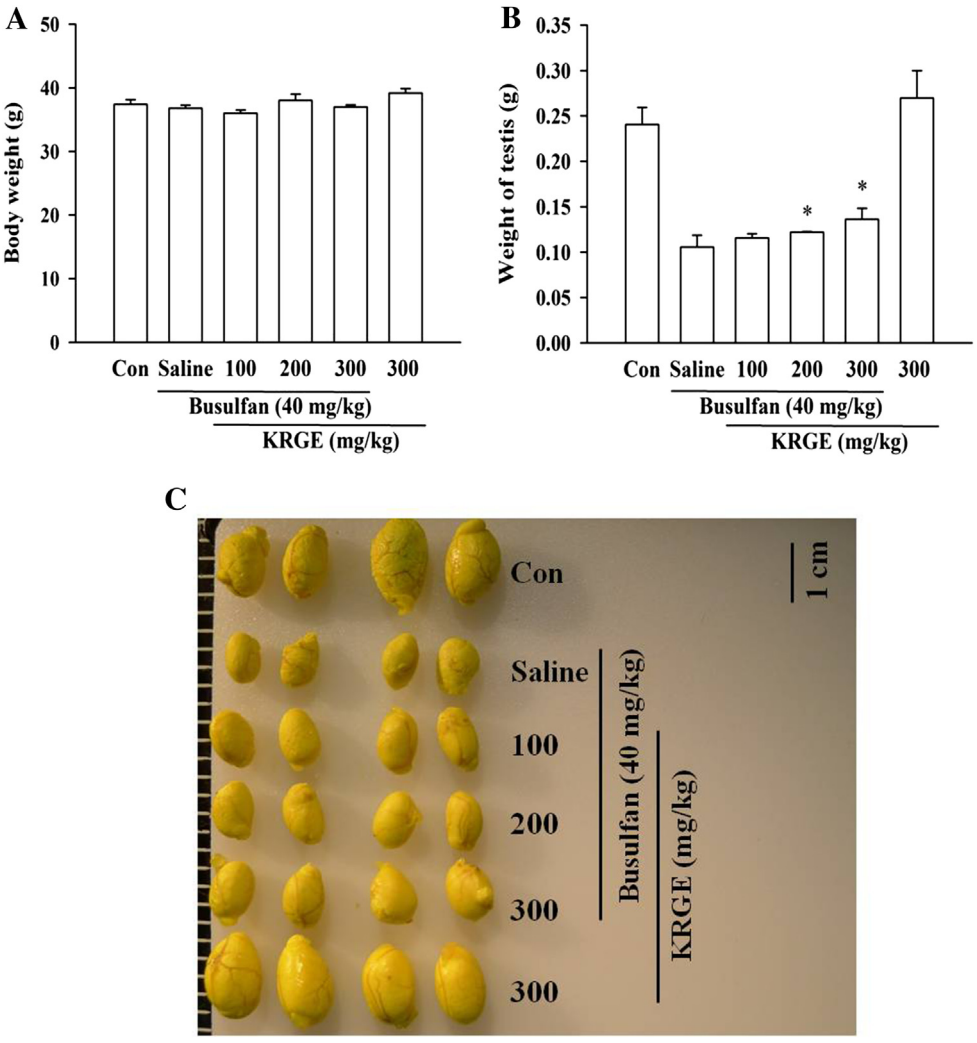

Fig. 2. Effects of Korean Red Ginseng extract (KRGE) against busulfan-induced damage on male body weight and on the size and weight of the testis. (A) Treatment with busulfan alone or with KRGE alone did not significantly affect the body weight of mice. (B and C) Treatment with busulfan alone induced a significant reduction of testis size and weight, whereas KRGE pretreatment before busulfan treatment partially restored testis weight ($n = 5$, $^*p < 0.05$, compared with busulfan alone). Treatment with KRGE alone did not affect testis weight. Con, control.

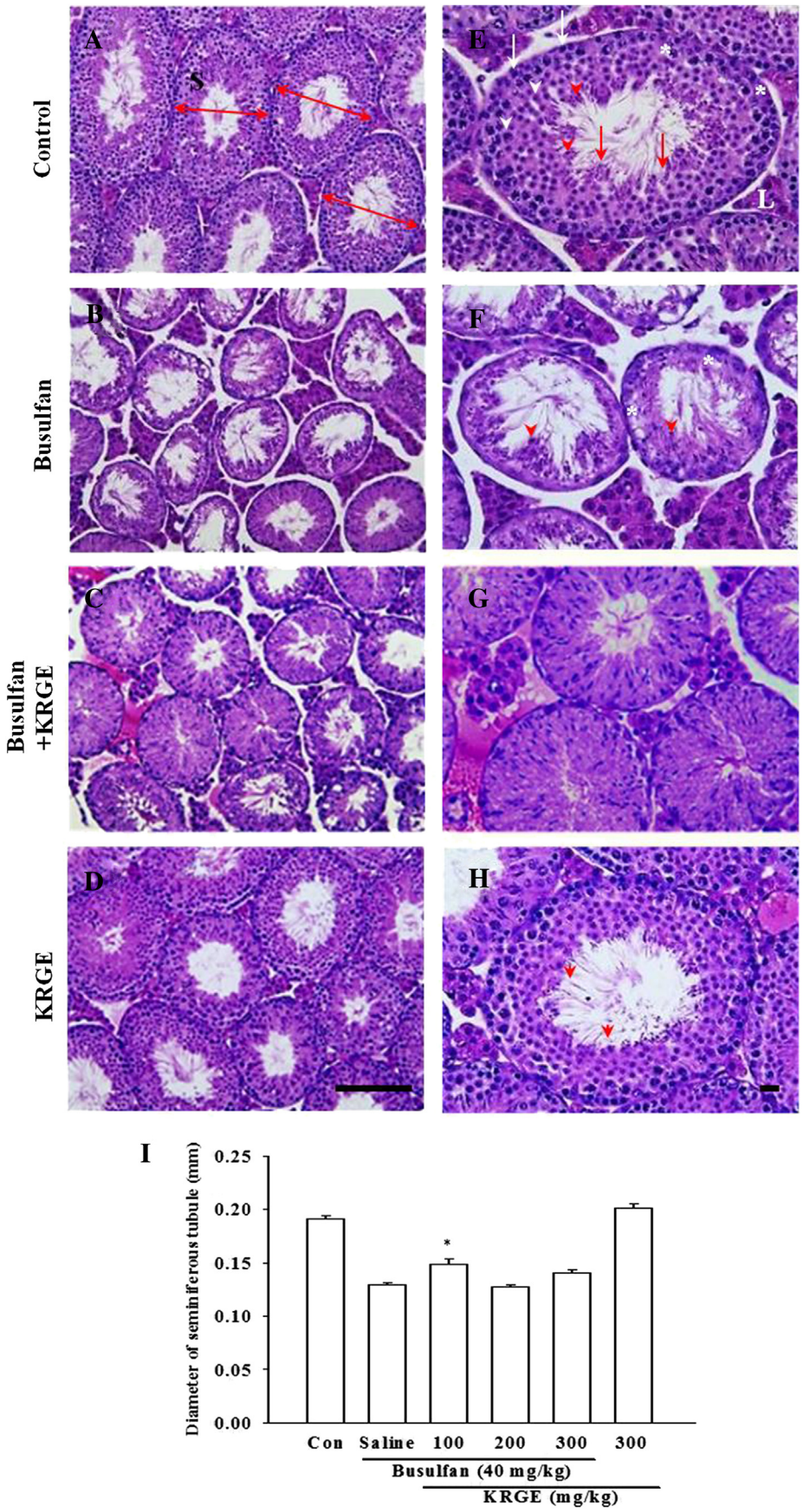

Fig. 3. Effects of Korean Red Ginseng extract (KRGE) on busulfan-induced histological damage in the seminiferous tubules. The testis from normal mice had regular spermatogonia (white arrows), primary/secondary spermatocytes (white arrowheads), spermatids (red arrows), spermatozoa (red arrowheads), and Sertoli cells (asterisks) in seminiferous tubules

manufacturer's protocol with minor modifications (Farmos Diagnostica, Oulunsalo, Finland) [16]. The Coat-A-Count procedure is a solid-phase RIA, based on a testosterone-specific antibody immobilized to the wall of a polypropylene tube. ^{125}I-labeled testosterone competes with testosterone in the experimental sample for antibody sites for a fixed time. The tube is then decanted, to separate bound from free testosterone, and counted in a gamma counter (Cobra; Hewlett Packard, Palo Alto, Califonia, USA). The amount of testosterone present in the experimental sample was determined from a calibration curve. All samples were run in triplicate, and two sets of testosterone standard were included in each assay.

2.8. Statistical analysis

All values are presented as the mean ± standard deviation and analyzed by one-way analysis of variance followed by Duncan multiple range test using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The significance of differences between the control and treatment values was determined using Student t test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of KRGE on busulfan-induced damage to the male reproductive system

Because it is known that administration of busulfan in mice causes male testicular damage [12], we first examined the effect of KRGE on changes of body weight and testis weight among the treatment groups. Although there were no significant changes in body weights among the treatment groups (Fig. 2A), compared with saline vehicle treatment, treatment with busulfan alone caused a significant decrease in testis weight (Fig. 2B and 2C). However, preadministration of KRGE before treatment with busulfan showed a dose-dependent tendency of attenuation of busulfan-induced testicular atrophy. Because the administration of busulfan alone affects the male reproductive system but not body weight, busulfan might selectively affect the testis. Next, we examined whether KRGE protects against histological damage induced by busulfan treatment. As shown in Fig. 3, in a representative histological analysis, the testis from normal mice had regular spermatogonia, primary/secondary spermatocytes, spermatids, spermatozoa, and Sertoli cells in the seminiferous tubules and Leydig cells between the tubules (Fig. 3A and 3E), whereas treatment with busulfan alone induced significant damage in the epithelium of the seminiferous tubule and narrowed the diameter of the seminiferous tubule. In other words, treatment with busulfan alone induced the irregular arrangements and reduced the diameter of the seminiferous tubule (Fig. 3B, 3F, and 3I). However, pretreatment with KRGE (100 mg/kg) before busulfan treatment restored busulfan-induced damage to the seminiferous tubule by increasing the epithelium of the seminiferous tubule and by widening the diameter of the seminiferous tubule (Fig. 3C, 3G, and 3I). Thus, it appears that KRGE partially restores busulfan-induced testicular damage. The KRGE-alone treatment had no histological effect on the testis (Fig. 3D, 3H, and 3I).

3.2. Effects of KRGE on busulfan-induced damage on sperm number and sperm motility

Because busulfan induced histological damage to the testis and KRGE attenuated busulfan-induced damage, we next examined the effects of KRGE against busulfan-induced damage on sperm number and sperm motility. As shown in Fig. 4A, treatment with busulfan alone decreased total numbers of sperm, but this decrease was not statistically significant from that with the vehicle control. Treatment with busulfan alone also decreased general or rapid sperm motility, but this was statistically insignificant; however, pretreatment with KRGE (100 mg/kg) significantly restored sperm number and sperm motility. Thus, KRGE attenuated busulfan-induced damage on sperm number and sperm motility.

3.3. Effects of KRGE on busulfan-induced decrease of male hormone concentration and litter size

We next examined the effects of KRGE on busulfan-induced changes in the level of the male hormone, testosterone, and litter size, because it was reported that male sterility was one of the adverse effects of busulfan in patients after cancer treatment. As shown in Fig. 5A, compared with the vehicle control, treatment with busulfan alone induced a decrease in testosterone concentration and litter size. However, preadministration of KRGE partially restored the busulfan-induced decrease of testosterone concentration and litter size (Fig. 5B). These results indicate that busulfan caused damage to the male reproductive system and that KRGE shows preventive effects against busulfan-induced damage to the male reproductive system.

4. Discussion

Cancer in various organs increases gradually and, although many patients recover from cancer, it is one of main causes of human death. Anticancer agents are useful for the treatment of cancer but these are associated with various adverse effects. The adverse effects of these anticancer agents on the reproductive system are especially severe, and they are one of the main causes of male sterility. Therefore, much research has been performed to develop functional foods or medicines to alleviate or reduce adverse effects induced by anticancer agents. Previous reports have suggested that the ginseng total saponin fraction attenuated cisplatin-induced nausea and vomiting in animal studies [9,10]. In the present study, we have shown that KRGE attenuated busulfan-induced damage to the male reproductive system. These results indicate that KRGE might have an efficacy for the attenuation of anticancer agent-induced adverse effects and that KRGE might be a novel candidate for developing drugs that reduce the adverse effects induced by busulfan.

However, pretreatment with KRGE did not completely prevent busulfan-induced damage to the male reproductive system. Therefore, if KRGE is used with other agents that attenuate the adverse effects of anticancer agents, it might be possible to achieve additive or synergic effects for reduction of the adverse effects of anticancer agents. In this study, it was also observed that compared with vehicle control, treatment with busulfan alone caused profound atrophy and histological damage to the testis (Fig. 2),

and Leydig cells (L) between the tubules (A and E). Compared with normal mice (A and E), animals that received treatment with busulfan alone showed significant damage in the epithelium (B and F) and reductions in the diameter of the seminiferous tubules (I), whereas pretreatment with 100 mg/kg KRGE before busulfan treatment significantly restored histological damage (C and G) and reduced the diameter of the seminiferous tubules (I). Treatment with KRGE alone did not affect the histological structure and diameter of seminiferous tubules (D, H, and I). The diameter of seminiferous tubule is indicated using the two-headed arrows in A ($n = 7$, $^*p < 0.05$, compared with busulfan alone). Scale bars = 100 μm. Con, control.

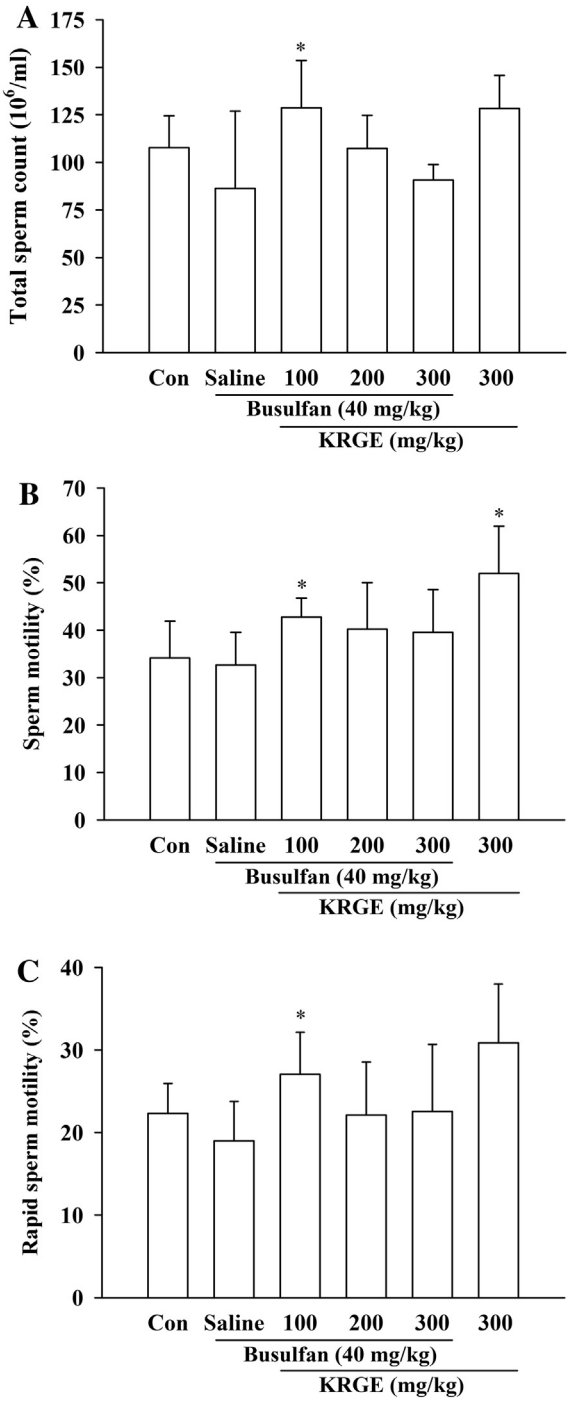

Fig. 4. Effect of Korean Red Ginseng extract (KRGE) on busulfan-induced changes in the male reproductive system. (A) Total sperm count. (B) Sperm motility. (C) Rapid sperm motility. Treatment of mice with busulfan alone caused a slight decrease in sperm number and motility, whereas pretreatment with 100 mg/kg KRGE before busulfan treatment significantly increased sperm number and motility ($n = 7$, $^*p < 0.05$, compared with busulfan alone). Con, control.

however, KGRE pretreatment attenuated busulfan-induced sperm number and motility reduction and decrease of serum testosterone (Figs. 4 and 5). In addition, we could not observe the dose-dependent effects of KGRE against busulfan-induced dysfunction of the male reproductive system. Further study is required to explain the lack of dose dependency of KGRE. Interestingly, busulfan did not induce complete sterility when we compared litter size with the control (Fig. 5). These results suggest a possibility that

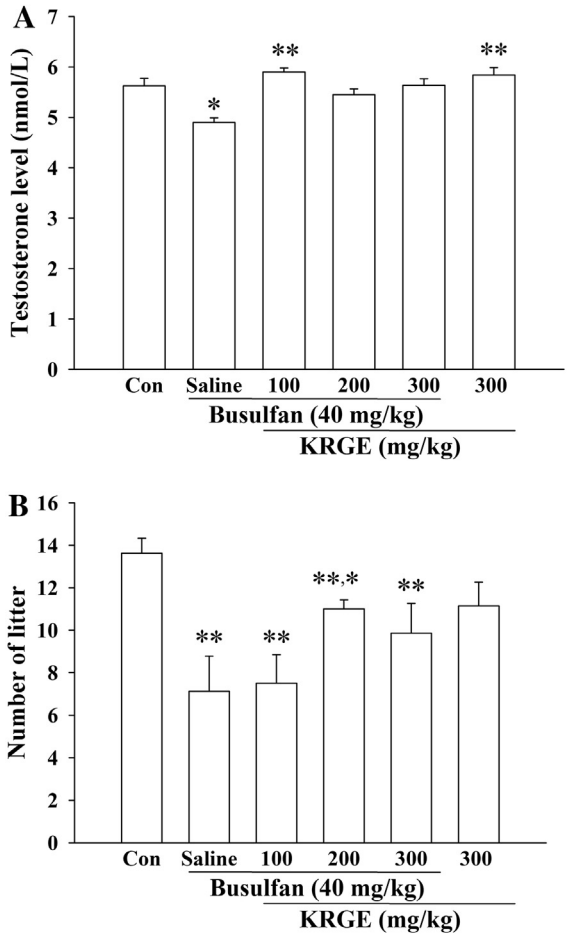

Fig. 5. Effect of Korean Red Ginseng extract (KRGE) on busulfan-induced decrease of serum testosterone concentration and litter size. (A) Treatment of mice with busulfan alone caused a decrease of serum testosterone concentration, whereas pretreatment with 100 mg/kg KRGE before busulfan treatment significantly restored serum testosterone concentration ($n = 7$, $^*p < 0.05$, compared with busulfan alone or 100 mg/kg KRGE before busulfan treatment). (B) Treatment of mice with busulfan alone caused a decrease of litter size compared with the control (Con), whereas pretreatment with 200 mg/kg KRGE before busulfan treatment significantly restored litter size ($n = 10$, $^{**}p < 0.05$, compared with busulfan alone).

compensating activities for the defense of the reproductive system might be playing a role after busulfan exposure.

The molecular mechanism of the attenuating effects of KRGE against busulfan-induced adverse effects on the male reproductive system can be speculated. A previous report showed that the attenuating effects of KRGE on cisplatin-induced nausea and vomiting are achieved through the inhibition of 5-HT_3 receptor channel activity [10]. In the present study, although we found that preadministration of KRGE exhibits protective effects against busulfan-induced damage to the male reproductive system, we could not explain the possible mechanism underlying KRGE-induced attenuation against busulfan-induced damage to the male reproductive system. Thus, although KRGE might be applied as a useful agent for prevention of damage induced by anticancer agents in the male reproductive system, further study is required to elucidate the molecular mechanism of KRGE against busulfan-induced adverse effects in the male reproductive system.

In addition, although we showed that KRGE attenuated busulfan-induced damage in the male reproductive system, we currently do not know which component(s) of ginseng alleviate the busulfan-induced adverse effects. Because KRGE is a mixture of various components of ginseng, the active ingredients in KRGE that

are responsible for the attenuation of busulfan-induced adverse effect in the male reproductive system are speculative. Currently, there are two representative components in KRGE that might contribute to the attenuation of busulfan-induced adverse effects. The first candidate is ginseng saponins. Ginseng saponins might alleviate busulfan-induced adverse effects, because they are known to have a variety of *in vitro* and *in vivo* pharmacological effects [2]. Furthermore, a few reports indicated that ginseng saponin exerts its effects on the male endocrine system and human sperm capacitance. Recent studies have also demonstrated that ginseng contains a G protein-coupled LPA receptor ligand, called *gintonin* [17]. Gintonin elicits $[Ca^{2+}]_i$ transient through the activation of the LPA receptor and regulates various Ca^{2+}-dependent ion channels and receptors [18]. Previous reports have indicated that LPA receptors are abundantly expressed in both female and male reproductive systems [19] and that LPA receptor-deficient animals showed pregnancy-related problems [20], indicating that LPA receptors in the reproductive systems play an important role in fertility. Thus, gintonin in KRGE might be one of the candidates for alleviation of busulfan-induced adverse effects in the male reproductive system. However, further work is required to identify the active ingredients of ginseng that are responsible for attenuating the effects of anticancer agents such as busulfan.

In summary, using an animal model of busulfan-induced adverse effects on the male reproductive system, we herein showed that busulfan elicited various adverse effects in the male reproductive system such as histological changes of the testis, reduction in the concentration of testosterone, and reduction in litter size. We showed that KRGE attenuated various busulfan-induced adverse effects in the male reproductive system. Finally, these novel findings provide new insights that KRGE could be applied as an agent for the attenuation or prevention of various adverse effects induced by anticancer agents.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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References

[1] Tyler VE. Herbal remedies. J Pharm Technol 1995;11:214–20.
[2] Nah SY, Kim DH, Rhim H. Ginsenosides: are any of them candidates for drugs acting on the central nervous system? CNS Drug Rev 2007;13:381–404.
[3] Shin TJ, Kim HJ, Kwon BJ, Choi SH, Kim HB, Hwang SH, Lee BH, Lee SM, Zukin RS, Park JH, et al. Gintonin, a ginseng-derived novel ingredient, evokes long-term potentiation through *N*-methyl-d-aspartic acid receptor activation: involvement of LPA receptors. Mol Cells 2012;34:563–72.
[4] Hwang SH, Shin EJ, Shin TJ, Lee BH, Choi SH, Kang J, Kim HJ, Kwon SH, Jang CG, Lee JH, et al. Gintonin, a ginseng-derived lysophosphatidic acid receptor ligand, attenuates Alzheimer's disease-related neuropathies: involvement of non-amyloidogenic processing. J Alzheimers Dis 2012;31:207–23.
[5] Hwang SH, Lee BH, Kim HJ, Cho HJ, Shin HC, Im KS, Choi SH, Shin TJ, Lee SM, Nam SW, et al. Suppression of metastasis of intravenously-inoculated B16/F10 melanoma cells by the novel ginseng-derived ingredient, gintonin: involvement of autotaxin inhibition. Int J Oncol 2013;42:317–26.
[6] Iwamoto T, Hiraku Y, Oikawa S, Mizutani H, Kojima M, Kawanishi S. DNA intrastrand cross-link at the 5′-GA-3′ sequence formed by busulfan and its role in the cytotoxic effect. Cancer Sci 2004;95:454–8.
[7] Karstens A, Kramer I. Chemical and physical stability of diluted busulfan infusion solutions. Eur J Hosp Pharm Sci Pract 2007;13:40–7.
[8] Shibata S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. J Korean Med Sci 2001;16:28–37.
[9] Kim JH, Yoon IS, Lee BH, Choi SH, Lee JH, Lee JH, Jeong SM, Kim SC, Park CK, Lee SM, et al. Effects of Korean Red Ginseng extract on cisplatin-induced nausea and vomiting. Arch Pharm Res 2005;28:680–4.
[10] Kim JH, Lee BH, Jeong SM, Nah SY. The inhibitory effects of Korean Red Ginseng saponins on 5-HT_{3A} receptor channel activity are coupled to anti-nausea and anti-vomiting action. J Ginseng Res 2009;29:37–43.
[11] Hwang SY, Kim WJ, Wee JJ, Choi JS, Kim SK. *Panax ginseng* improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. BJU Int 2004;94:663–8.
[12] Zohni K, Zhang X, Tan SL, Chan P, Nagano MC. The efficiency of male fertility restoration is dependent on the recovery kinetics of spermatogonial stem cells after cytotoxic treatment with busulfan in mice. Hum Reprod 2012;27:44–53.
[13] Connolly CM, Dearth AT, Braun RE. Disruption of murine Tenr results in teratospermia and male infertility. Dev Biol 2005;278:13–21.
[14] Oh MS, Yang WM, Chang MS, Park W. Kim do R, Lee HK, Kim WN, Park SK. Effects of *Rubus coreanus* on sperm parameters and cAMP-responsive element modulator (CREM) expression in rat testes. J Ethnopharmacol 2007;114:463–7.
[15] George M, Rainey MA, Naramura M, Ying G, Harms DW, Vitaterna MH, Doglio L, Crawford SE, Hess RA, Band V, et al. Ehd4 is required to attain normal prepubertal testis size but dispensable for fertility in male mice. Genesis 2010;48:328–42.
[16] Monath JR, McCullough DL, Hart LJ, Jarow JP. Physiologic variations of serum testosterone within the normal range do not affect serum prostate-specific antigen. Urology 1995;46:58–61.
[17] Choi SH, Shin TJ, Lee BH, Hwang SH, Kang J, Kim HJ, Park CW, Nah SY. An edible gintonin preparation from ginseng. J Ginseng Res 2011;35:471–8.
[18] Hwang SH, Shin TJ, Choi SH, Cho HJ, Lee BH, Pyo MK, Lee JH, Kang J, Kim HJ, Park CW, et al. Gintonin, newly identified compounds from ginseng, is novel lysophosphatidic acids-protein complexes and activates G protein-coupled lysophosphatidic acid receptors with high affinity. Mol Cells 2012;33:151–62.
[19] Rivera R, Chun J. Biological effects of lysophospholipids. Rev Physiol Biochem Pharmacol 2008;160:25–46.
[20] Ziecik AJ, Waclawik A, Bogacki M. Conceptus signals for establishment and maintenance of pregnancy in pigs—lipid signaling system. Exp Clin Endocrinol Diabetes 2008;116:443–9.