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Effects of Korean Red Ginseng Extract on Cisplatin-Induced Nausea and Vomiting

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Ginseng, the root of *Panax ginseng* C.A. Meyer, is well known as a tonic medicine for restoring and enhancing human health. In traditional medicine, ginseng is utilized for the alleviation of emesis, which includes nausea and vomiting. However, it has not yet been demonstrated whether ginseng exhibits *in vivo* anti-nausea and anti-vomiting properties. In this study, we examined the anti-emetic effect of Korean red ginseng total extract (KRGE) on cisplatin-induced nausea and vomiting using ferrets. Intraperitoneal administration (i.p.) of cisplatin (7.5 mg/kg) induced both nausea and vomiting with one-hour latency. The episodes of nausea and vomiting reached a peak after 1.5 h and persisted for 3 h. Treatment with KRGE *via* oral route significantly reduced the cisplatin-induced nausea and vomiting in a dose-dependent manner. The anti-emetic effect was 12.7 ± 8.6, 31.8 ± 6.9, and 67.6 ± 4.0% with doses of 0.3, 1.0, and 3.0 g/kg of KRGE, respectively. Pretreatment with KRGE *via* oral route 1 and 2 h before cisplatin administration also significantly attenuated the cisplatin-induced nausea and vomiting. However this did not occur with a pretreatment 4 h before cisplatin administration. These results are supportive of KRGE being utilized as an anti-emetic agent against nausea and vomiting caused by chemotherapy (i.e. cisplatin).

Key words: *Panax* ginseng, Korean red ginseng extract, Cisplatin, Emesis, Anti-emesis

INTRODUCTION

Traditionally, ginseng, the root of *Panax ginseng* C.A. Meyer, has been used as a tonic herbal medicine for the restoration and enhancement of human health, such as maintenance of visceral functions, tranquilization, protection against diseases and enhancement of memory function and longevity. Moreover, in traditional medicine ginseng has been used for the alleviation of emesis, which includes nausea and vomiting (Abe, 1965). Administration of anticancer agents in the course of chemotherapy or radiotherapy as well as general anesthesia induces nausea and vomiting. These side effects limit the absorption of the drugs and lowers the efficacy of the administered drugs in the human body (Sung, 1996). Recent studies have shown that the development of agent(s) controlling emesis, including nausea and vomiting, is one of the major research topics in current clinical research.

In this study, we investigated the effect of Korean red ginseng total extract (KRGE) on cisplatin-induced nausea and vomiting using ferrets (Kan *et al.*, 2002). Since it has not yet been demonstrated *in vivo* whether KRGE exhibits anti-nausea and anti-vomiting properties. In this study, we used cisplatin and ferrets because: (1) cisplatin is an anticancer agents that has been widely used for the induction of nausea and vomiting in veterinary experiments (King, 1990) and (2) rodents such as rat and mouse are not sensitive to emetogen, because they do not have a vomiting center in their central nervous system. Whereas ferrets are sensitive to emetogen and widely used for the evaluation of anti-emetic agents, in spite of the fact that they are expensive animals (King, 1990; Rudd *et al.*, 1996; Hawthorn *et al.*, 1998; Kan *et al.*, 2002). In this study, it was considered to be statistically significant that treatment of KRGE *via* oral route reduced the cisplatin-

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induced nausea and vomiting with a dose- and time dependent manner. Pretreatment of KRGE *via* oral route 1 and 2 hours before cisplatin administration also significantly attenuated the cisplatin-induced nausea and vomiting. However this did not occur with a pretreatment 4 hours before cisplatin administration. These results are supportive of KRGE being utilized as an anti-emetic agent against nausea and vomiting caused by chemotherapy (i.e. cisplatin).

MATERIALS AND METHODS

Materials

KRGE was obtained from the Korea Ginseng Cooperation (Daejeon, Korea). To acquire KRGE, dried Korean red ginseng roots (6 years old) were pulverized into a powder and passed through a 40-mesh sieve. The powdered Korean red ginseng (1 kg) was extracted 4 times in 10,000 mL of hot water (80-83°C) for 8 h. The extraction solution was cooled down to 25°C and filtered with a 80-mesh membrane filter. The filtrate was cooled down again to 10°C and filtered with a 60-mesh filter and then centrifuged at 7,500 rpm for 20 minutes. The resulting supernatant consisted of 36% water and 64% solid ginseng extract. It was kept at 55-65°C for 5 days. The used hot water extraction procedure for Korean red ginseng root is known to contain carbohydrates, proteins, and ginsenosides. This procedure minimizes the loss of all the various ginseng components, whereas the ethanol or methanol extraction procedure can retain more ginsenoside fraction than the hot water extraction procedure. However a greater amount of the other components, would be lost, which is undesirable in this case. Cisplatin and other chemical agents were obtained from Sigma (St. Louis, MO).

Animals

The experiments were carried out with castrated male ferrets (*Mustela putorious furo*, 1.0-1.5 kg) bred at Japan SLC, Inc. Prior to the experiments, they were housed communally at 22 ± 2°C with the lights on between 07:00 and 19:00. The relative humidity was 50 ± 5% and they had free access to ferret food (Ferret DietR 5003, PMIR Feeds, U.S.A.) and water.

Drug administrations

To facilitate the intraperitoneal administration (i.p.) of drugs, the animals were lightly anaesthetized with 5% halothane (carrier: N_2 80%, O_2 20%). Cisplatin and KRGE were freshly dissolved in saline (0.9% w/v). Various dosages of KRGE were orally administered in order to determine the dose- and time response effect of KRGE against cisplatin-induced emesis. *Via* a cannula a volume of 10 mL was administered 60 min before cisplatin (7.5 or 10 mg/kg) was administered intraperitoneally over a 2 min period. (Sam *et al.*, 2003). The control group received only saline orally. The animals were transferred to individual observation cages for the assessment of nausea and vomiting. All animals regained consciousness within 1-5 min after discontinuation of the anesthesia. To determine the time-dependent effects of KRGE against cisplatin-induced emesis, KRGE was orally pre-administered with 3 g/kg at 1, 2, and 4 h before cisplatin (7.5 or 10 mg/kg) administration.

Measurements of nausea and vomiting

Animal behavior was recorded with a remote video camera and analyzed at the end of the experiments. The symptoms of nausea and vomiting were characterized by rhythmic abdominal contractions that were either associated with the oral expulsion of liquid or solid material from the gastrointestinal tract (i.e. vomiting) or not associated with the passage of material (i.e. nausea). Every 30 min. interval the total number of episodes of nausea and vomiting were marked (Lucot, 1989).

Data analysis

In each animal, the latency to nausea or vomiting following the administration of the respective emetogen was measured. The total sum of episodes of nausea and vomiting were calculated for the duration of the experiments. It was then expressed as the mean ± S.E.M. % inhibition, by KRGE administration, of cisplatin (7.5 or 10.0 mg/kg)-induced nausea and vomiting. The mean ± S.E.M. % inhibition was determined as follow: % inhibition = $100 \times [1 - (\text{number of nausea and vomiting by the KRGE treated animals/number of nausea and vomiting by the control group})$. The data was analyzed using Dunnett's multiple comparison tests to compare each treatment group with the control group. A value of $P < 0.05$ was considered statistically significant in the analysis.

RESULTS AND DISCUSSION

To see whether traditional utilization of ginseng for alleviation of nausea and vomiting is also applicable in modern medicine, we investigated the *in vivo* effect of KRGE on cisplatin-induced emesis using ferrets. In this study, we first determined the required dosage of cisplatin in order to induce nausea and vomiting. Administration of less than 5.0 mg/kg cisplatin intraperitoneally did not induce vomiting and nausea (data not shown). As shown in Fig. 1A, after we increased the dosage of cisplatin (7.5 mg/kg, i.p.), the ferrets began to show nausea and vomiting episodes. This occurred 60 min after the cisplatin administration. These episodes reached a peak after 1.5 h and persisted for 3 h. As a next step, we investigated

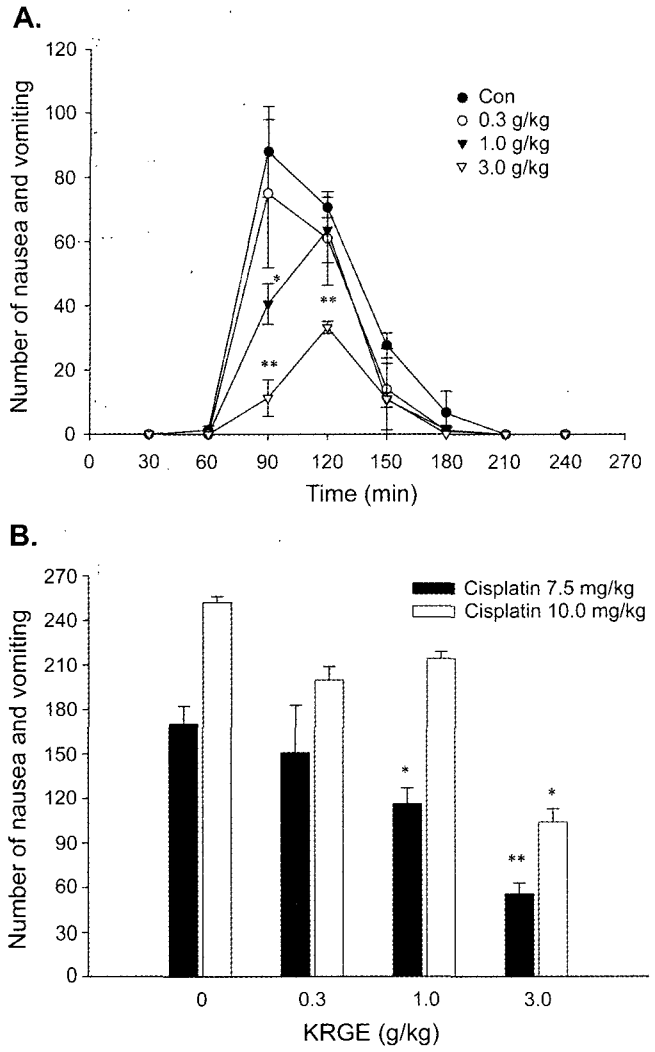

Fig. 1. Dose-dependent effects of KRGE on the cisplatin-induced nausea and vomiting. Control vehicle animal groups were administered with saline, whereas KRGE-treated animal groups were orally administered with increasing dosages of KRGE before cisplatin was given. After 60 min, cisplatin (7.5 mg/kg, i.p.) was administered to both groups. The nausea and vomiting induced by cisplatin administration was recorded for every 30 min interval during a total of 4 hours. **A**. The dose-dependent effects of KRGE in the cisplatin-induced nausea and vomiting. Administration of cisplatin starts to induce nausea and vomiting after a 1 h latency, but treatment of KRGE reduces the cisplatin-induced nausea and vomiting with a dose-dependent manner. **B**. The histograms shows the total sums of nausea and vomiting for 4 hours, which was induced by cisplatin (7.5 or 10 mg/kg) administration, with the different concentrations of KRGE. Data represent the mean ± S.E.M. *$p<0.05$, **$p<0.01$ compared with saline treatment alone, n=5-6 in each data point.

the effect of KRGE on the cisplatin-induced nausea and vomiting. When we administered 0.3 g/kg KRGE *via* oral route, we observed only slight inhibition in cisplatin (7.5 mg/kg, i.p.)-induced nausea and vomiting (Fig. 1A). However, the oral administration of 1.0 and 3.0 g/kg KRGE significantly reduced the cisplatin-induced nausea and vomiting (Fig. 1A). Thus, anti-emetic effect of KRGE was dose-dependent. Anti-emetic effect of KRGE was 11.7 ± 31.6, 31.8 ± 10.7, and 67.3 ± 7.2% at dosages of 0.3, 1.0, and 3.0 g/kg of KRGE, respectively (Fig. 1B). However, when we increased the administered dosage of cisplatin to 10 mg/kg, we only observed a significant anti-emetic effect of KRGE at dosage of 3.0 g/kg by 58.7 ± 9.0% (Fig. 1B). These results indicate that KRGE attenuates the cisplatin-induced nausea and vomiting in a dose-dependent manner, meaning that a higher dosage of KRGE might be required when cisplatin dosage is increased.

Furthermore, we investigated the time-dependent effect of KRGE on the cisplatin (7.5 mg/kg, i.p.)-induced nausea and vomiting. For this part, we administered KRGE (3.0 g/kg, p.o.) to ferrets 1, 2, and 4 hours before the cisplatin treatment. As shown in Fig. 2B, administration of KRGE *via* oral route (3.0 g/kg) 1 and 2 h before cisplatin administration significantly attenuated cisplatin-induced nausea and vomiting by 73.2 ± 17.5 and 59.1 ± 24.0%. However administration of KRGE (3.0 g/kg, p.o.) 4 h before cisplatin administration showed only a slight attenuation on the cisplatin-induced nausea and vomiting by 23.4 ± 16.9%. When we increased the cisplatin dosage to 10 mg/kg, we could also observe similar time-dependent anti-emetic effects of KRGE, but the effect of KRGE was less potent than that obtained while using a dosage of 7.5 mg/kg cisplatin (Fig. 2B). Thus, anti-emetic effect of KRGE was 37.6 ± 18.6, 31.2 ± 16.5, and 5.6 ± 4.0% at 1, 2, and 4 h before cisplatin administration, respectively. These results indicate that KRGE attenuates cisplatin-induced nausea and vomiting with a time-dependent manner.

Nausea and vomiting are one of the most frequently reported side effects by patients whom are given general anesthesia before and after an operation (Gale, 1995). Moreover, most of the cancer patients whom are treated with chemotherapy or radiotherapy also experience nausea and vomiting (Sung, 1996). This study was designed to evaluate the anti-emetic effect of KRGE and we performed *in vivo* experiments to determine whether KRGE has an anti-emetic effect in ferrets. We found that KRGE attenuates the cisplatin-induced nausea and vomiting with a dose- and time-dependent manner. Moreover, we evidenced the traditional ginseng effect against nausea and vomiting. These results are supportive of ginseng being used as an agent attenuating nausea and vomiting.

Recent reports evidenced that the 5-hydroxytryptamine type$_{3A}$ (5-HT$_{3A}$) receptor is present in both the central and peripheral nervous systems (Ortells and Lunt, 1995). 5-HT$_{3A}$ receptor is involved in physiological and pathological processes that mainly mediate nausea and vomiting. This administration of 5-HT$_3$ receptor antagonists attenuate and/or block the incidence and severity of general

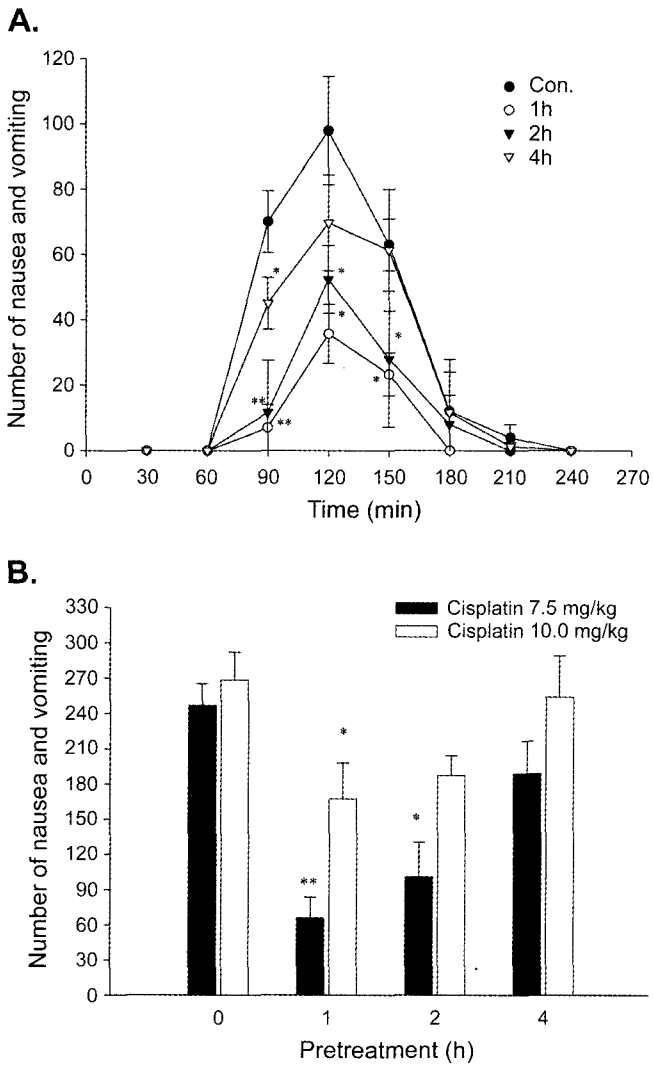

Fig. 2. Time-dependent effect of KRGE on cisplatin-induced nausea and vomiting. **A.** KRGE (3.0 g/kg) was orally pre-administered with the indicated time (● control; ○, 1 h; ▼, 2 h; ▽, 4 h) before cisplatin (7.5 mg/kg) treatment, whereas control vehicle animal groups were only administered saline. The administration of KRGE 1 and 2 before cisplatin reduced cisplatin-induced nausea and vomiting. However this did not occur with a pretreatment 4 hours before cisplatin administration. **B.** The histograms show the total sum of nausea and vomiting for 4 hours, which was induced by cisplatin (7.5 or 10 mg/kg) administration after oral pre-administration of KRGE (3.0 g/kg) with the indicated time before cisplatin treatment. Data represent the mean ± S.E.M. *$p<0.05$, **$p<0.01$ compared with saline treatment alone, n=5-6 in each data point.

anesthesia- or chemotherapy- or radiotherapy-induced nausea and vomiting. These antagonists are currently used for clinical treatment (Perez and Gandara, 1992; Chevallier, 1993; Polati, *et al.*, 1997; Fortney *et al.*, 1998). However, they have adverse effect on the cardiac function, which limits their routine use (Sung, 1996). In contrast, ginseng has mild pharmacological effects and has no known side effects even while using high dosages. It does not exhibit weak hemolytic activity, when injected into the peritoneum of mice (Kaku *et al.*, 1975). This means that it KRGE could be used to prevent or attenuate nausea and vomiting, even when injected intraperitoneally.

The question remains, what are the major active ingredient(s) of KRGE in the anti-emetic effect on ferrets? A chain of evidence showed that the main molecular components responsible for the actions of ginseng are ginsenosides, which are also known as ginseng saponins. Ginsenosides have a four-ring, steroid-like structure with sugar moieties attached. About 30 different forms have been isolated and identified from the roots of *Panax ginseng*. On the other hand, 5-HT_{3A} receptor belongs to the superfamily of ligand-gated ion channel receptors that share the structural similarity with other ligand-gated ion channels like nicotinic acetylcholine receptors (Ortells and Lunt, 1995). This receptor mediates the rapid and transient excitatory synaptic transmission, as do nicotinic acetylcholine receptors. Recent reports showed that ginsenosides affect ligand-gated ion channel activity. In cells expressing nicotinic acetylcholine receptors, such as bovine chromaffin cells, ginsenosides inhibit acetylcholine-stimulated Na^+ influx (Kudo *et al.*, 1998) and Choi *et al.* (2002), Lee *et al.* (2004), and Sala *et al.* (2002) showed that they inhibit acetylcholine- and 5-HT-induced inward currents in *Xenopus* oocytes expressing several subtypes of neuronal and $\alpha\beta\gamma\sigma$ muscle-type nicotinic acetylcholine or 5-HT_{3A} receptors. Thus, these reports support that ginsenosides in KRGE might play an important role in the anti-emetic effects of ginseng, as shown in this study. However, we could not exclude the possibility that other components, besides ginsenosides, are also involved in KRGE-induced anti-emetic effects and further studies will be needed to clarify the role of ginsenosides and other components in KRGE-induced anti-emetic efficacy.

In summary, we demonstrated that KRGE attenuates cisplatin-induced nausea and vomiting with a dose- and time-dependent manner in ferrets. These results are supportive of KRGE being utilized as an anti-emetic agent against chemotherapy (i.e. cisplatin)-caused nausea and vomiting.

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