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Ginsenoside rich fraction of *Panax ginseng* C.A. Meyer improve feeding behavior following radiation-induced pica in rats

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ABSTRACT

Panax ginseng is an indigenous medicinal herb and has traditionally been used among Asian population for relief of many human ailments. We investigated the prophylactic role of Korean *P. ginseng* extract (KG) against X-ray irradiation-induced emesis in an acute rat pica model. Rats were treated with KG (12.5, 25, 50 mg/kg orally at −48, −24 and 0 h) prior to X-ray irradiation (6 Gy), and intake of kaolin and normal food and body weight changes examined as an index of the acute emetic stimulus. Levels of serotonin in small intestine tissue were assessed and histopathology of gastric tissue, small intestine and colon examined specific staining. Pre-treatment with KG (12.5 and 25 mg/kg) reduced X-ray irradiation-induced kaolin intake at 24 h. Normal food intake was improved in rats treated with 25 mg/kg KG. The anti-emetic effect of KG was further confirmed on the basis of serotonin release, histopathological findings. Our findings collectively indicate that KG protects against X-ray irradiation-induced acute pica to a moderate extent, leading to improved feeding behavior in rats.

1. Introduction

Gastrointestinal symptoms, such as nausea and vomiting, are common in patients receiving cancer radiotherapy [1]. However, radiotherapy-induced emesis can impair quality of life and lead to discontinuation of potential therapy. While radiotherapy is established as one of the most effective modalities of cancer treatment, several clinical trials to date have shown that subjects receiving whole-body or upper abdominal irradiation often undergo acute episodes of vomiting [2].

Currently available antiemetics, including ondansetron, granisetron, and metoclopramide, either alone or in combination with glucocorticosteroids, such as dexamethasone, are prescribed for the prevention of emesis during highly emetogenic radiation therapy [3]. These drugs are potential dopamine D2, tachykinin NK1 or $5HT_3$ receptor antagonists that effectively prevent vomiting in chemotherapy and radiotherapy patients [4]. However, a significant number of cancer patients continue to experience vomiting or nausea, despite administration of anti-emetic drugs [5]. Furthermore, the available medications are concurrently associated with various side-effects, including headache, constipation, restlessness, and dystonic reactions. Combination therapy with benzodiazepines or antihistamines is necessary to avoid these additional side-effects, but often results in sedation [6].

Panax ginseng C.A. Meyer (*P. ginseng*), commonly known as 'Korean ginseng' is one of the most popular adaptogens among medicinal plants with a long history of traditional use [7]. Recent research has led to the identification of ginsenosides as the major active compounds with a wide range of pharmaceutical activities, including anti-inflammation, anti-stress, radioprotection, anti-fatigue, immunomodulation and anti-tumor properties [8–12]. Although synthetic anti-emetic drugs have

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yielded the highest protective activity to date, typically, these compounds are more toxic than naturally occurring remedies. In general, the use of these anti-emetic agents results in behavioral toxicity [13]. Hence, the search for alternative sources, including bioactive principles of herbal origin, is a continuing focus of research worldwide. We recently reported an anti-emetic effect of KG in a cisplatin-induced rat-pica model. To our knowledge, no data on the effects of *P. ginseng* on irradiation-induced emesis are documented in the literature. In the current study, we have established that KG exerts anti-emetic and protective activities against X-ray irradiation-induced emesis in a rat pica model.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (Orientbio, Gyeonggido, Korea) weighing between 100 and 120 g were used in this study. Rats were housed in standard polypropylene transparent cages (one rat/cage) covered with metallic grids (Jeung Bio & Plant, Seoul, Korea) under environmentally controlled conditions (temperature, 22 ± 2 °C; humidity, 55–60%) with a 12 h light: 12 h dark cycle (light on at 09:00 and 21:00 h). Animals were fed commercial standard chow (Samtako, Osan, Korea) and tap water ad libitum. For X-ray irradiation-induced pica, kaolin pellets were placed in adjacent separate compartments in a divided food hopper to ensure constant ease of access throughout the experiment. Sterile metal grills were used inside the cage to prevent free access to wood shavings or stool. X-ray irradiation-induced pica experiments were designed and performed in strict accordance with the regulations for laboratory animal care (NIH publication No. 85-23, revised 1985) approved by the Institutional Animal Care and Use Committee of Daejeon University (DJUARB 2010-039).

2.2. Preparation of KG and HPLC analyses

Korean *P. ginseng* root was obtained from Ginseng Nonghyup (Keum-san, Korea). Sliced ginseng roots (1.2 kg) were boiled in 6.5 l of water for 100 min at 120 °C using a high-speed automatic non-pressure earthen pot (Kyung-Hee Co-operation, Seoul, Korea), and the extraction procedure repeated with 4.5 l of water. KG was further centrifuged for 30 min at 1500 rpm and the supernatant lyophilized using a vacuum-freeze drying system, followed by storage at −40 °C. The extract yield was 7.1–8%. Limulus amebocyte lysate (LAL) assay (Endotoxin ELISA Kit, antibodies-online Inc., USA) was performed to determine the endotoxin levels. Evaluation of nitrite and nitrate (NOx) release (Cayman Chemical Nitrate/Nitrite Assay Kit, USA) in a mouse leukemic monocyte macrophage cell line and rat peritoneal macrophage examination were additionally performed.

The amounts of crude total saponin, protopanaxadiol and protopanaxatriol ginsenosides in KG were estimated using the High Performance Liquid Chromatographic (HPLC) system consisting of a Waters Alliance 2695 HPLC pump, Waters Alliance 2695 Auto sampler, and Waters 996 PDA (United States, Connecticut). HPLC was performed under conditions similar to those described in a previous report [14].

2.3. Preparation of kaolin pellets

Kaolin ($H_2Al_2Si_2O_8.H_2O$) was prepared according to a previously described technique with slight modifications [16]. Briefly, pharmacological-grade kaolin (Samchun Pure Chemical Co., LTD. Pyeongtaek, Korea) was mixed with 2% acacia or Gum Arabic in double-distilled water to form a thick paste. The paste was rolled and cut into small pieces resembling regular rat food pellets. Pellets were dried completely at room temperature for 4–5 days, and stored under moisture-free sterile conditions.

2.4. Animal grouping and X-ray radiation

After a one-week acclimatization period, 72 rats were housed separately in cages and familiarized with the adaption procedures before the start of pica experiments. In addition to routine rat chow and tap water, a measured quantity of kaolin (1–2 g) pellets was filled in separate containers for 4 days prior to the pica experiment to allow psychological adaptation of rats to its presence. After 4 days of habituation, rats were subjected to X-ray irradiation (6 Gy). The experimental irradiation dose was fixed on the basis of kaolin consumption under our laboratory conditions. Irradiation doses of 2 Gy, 4 Gy and 6 Gy were used in the preliminary study.

Psychologically adapted rats were divided into six groups: normal (radiation naïve control), X-ray irradiation-induced pica (6 Gy), and KG-12.5, KG-25, KG-50 and Met-50 treatment groups. At −48 h, −24 h and 0 h, each group was orally administered water (normal and irradiation groups) or three doses of KG (12.5, 25, and 50 mg/kg body weight) or the standard reference antiemetic, metoclopramide HCl (Met 50 mg/kg).

Two hours after administration of the final dose of water, KG or Met, all except the normal group were subjected to X-ray irradiation. The whole-body X-ray irradiation dose was 6 Gy at a dose rate of 3 Gy/min using a linear accelerator (Siemens Medical Solutions, Erlangen, Germany) with a field size 36∗36 cm and distance from photon irradiation to the object of 100 cm (source axis distance). Linear accelerated energy (6 MV) was exposed in both the front/back directions in an acryl box (30∗30∗5 cm), and animals were allowed free access to measured quantities of normal, water and kaolin diets.

2.5. Evaluation of pica, anorexia and body weight reduction

To estimate total kaolin and normal food consumption for each experimental day, kaolin and food remaining from the previous day were collected, including that spilled outside or inside containers. Body weight was assessed at 24 h.

2.6. Estimation of serotonin (5-Hydroxytryptamine, 5-HT) in small intestine

Quantitative determination of 5-Hydroxytryptamine (5HT) in the small intestine was performed using the immunoassay kit (USCN Life Science & Technology Co., Ltd, China) operating instructions. Briefly, microtiter plates pre-coated with an antibody specific for 5HT were loaded with standards or test samples (100 μl) with a biotin-conjugated polyclonal antibody preparation specific for 5HT. Avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and