Cancer Chemother Pharmacol (2005) 56: 63–69
DOI 10.1007/s00280-004-0956-1

ORIGINAL ARTICLE

**Sangeeta Mehendale · Han Aung · Anbao Wang
Jun-Jie Yin · Chong-Zhi Wang · Jing-Tian Xie
Chun-Su Yuan**

American ginseng berry extract and ginsenoside Re attenuate cisplatin-induced kaolin intake in rats

Received: 9 August 2004 / Accepted: 12 November 2004 / Published online: 25 March 2005

Abstract *Purpose*: Cisplatin, a chemotherapeutic agent, causes significant nausea and vomiting. It is postulated that cisplatin-induced oxidant stress may be responsible for these symptoms. We tested whether pretreatment with American ginseng berry extract (AGBE), an herb with potent antioxidant capacity, and one of its active antioxidant constituents, ginsenoside Re, could counter cisplatin-induced emesis using a rat pica model. *Methods*: In rats, exposure to emetic stimuli such as cisplatin causes significant kaolin intake, a phenomenon called pica. We therefore measured cisplatin-induced kaolin intake as an indicator of the emetic response. Rats were pretreated with vehicle, AGBE (dose range 50–150 mg/kg, IP) or ginsenoside Re (2 and 5 mg/kg, IP). Rats were treated with cisplatin (3 mg/kg, IP) 30 min later. Kaolin intake, food intake, and body weight were measured every 24 h for 120 h. Additionally, the free radical scavenging activity of AGBE was measured in vitro using ESR spectroscopy. *Results*: A significant dose-response relationship was observed between increasing doses of pretreatment with AGBE and reduction in cisplatin-induced pica. Kaolin intake was maximally attenuated by AGBE at a dose of 100 mg/kg. Food intake also improved significantly at this dose ($P < 0.05$). Pretreatment with ginsenoside Re (5 mg/kg) also decreased kaolin intake ($P < 0.05$). In vitro studies demonstrated a concentration-response relationship between AGBE and its ability to scavenge superoxide and hydroxyl radicals. *Conclusion*: Pretreatment with AGBE and its major constituent, Re, attenuated cisplatin-induced pica, and demonstrated potential for the treatment of chemotherapy-induced nausea and vomiting. Significant recovery of food intake further strengthens the conclusion that AGBE may exert an antinausea/antiemetic effect.

Keywords American ginseng · Berry · Ginsenoside Re · Herbal medicine · Cisplatin

S. Mehendale · H. Aung · A. Wang · C.-Z. Wang
J.-T. Xie · C.-S. Yuan
Tang Center for Herbal Medicine Research,
The Pritzker School of Medicine, University of Chicago,
5841 S. Maryland Avenue, MC 4028,
Chicago, IL 60637, USA

S. Mehendale · H. Aung · A. Wang · C.-Z. Wang
J.-T. Xie · C.-S. Yuan
Departments of Anesthesia and Critical Care,
The Pritzker School of Medicine, University of Chicago,
Chicago, IL 60637, USA

C.-S. Yuan (✉)
Committee on Clinical Pharmacology and Pharmacogenomics,
The Pritzker School of Medicine, University of Chicago,
Chicago, IL 60637, USA
E-mail: cyuan@airway.uchicago.edu
Tel.: +1-773-7021916
Fax: +1-773-8340601

J.-J. Yin
Center for Food Safety and Applied Nutrition,
FDA, College Park, MD, 20740, USA

Introduction

Treatment with cancer chemotherapy and radiotherapy causes unpleasant symptoms such as nausea, vomiting and abdominal discomfort [1–3]. These adverse effects are significant as they cause increased patient morbidity and anticipatory symptoms in the subsequent treatment cycles [1]. The pathophysiology of these symptoms has been partly attributed to oxidant injury to the intestinal epithelium [4, 5]. The mucosal injury results in excessive serotonin release from the enterochromaffin cells that could mediate the gastrointestinal adverse effects of chemotherapy and radiotherapy [6–10]. Since oxidant injury to the gut may be the primary event responsible for the gastrointestinal symptoms following chemotherapy or radiotherapy, we hypothesized that pretreatment with an antioxidant should ameliorate these symptoms.

In this study, we tested the efficacy of an herbal antioxidant, American ginseng berry extract (AGBE), in reducing chemotherapy-induced nausea and vomiting. We used a rat pica model of simulated emesis, that responds to emetic stimuli by increasing consumption of non-nutritive substances such as clay or kaolin [11–13]. The simulated emesis model is further validated by the observation that treatment with antiemetics reduces pica [14, 15]. The chemotherapeutic agent, cisplatin, which induces significant nausea and vomiting in humans and in other animals, causes pica in rats [9, 15–17]. We therefore measured the effect of AGBE pretreatment on cisplatin-induced pica in rats. We also sought to determine if pretreatment with an active constituent of AGBE, ginsenoside Re, affected pica in cisplatin-treated rats. In addition, the ability of AGBE to scavenge superoxide and hydroxyl radicals was measured in vitro using electron spin resonance (ESR) spectroscopy.

Materials and methods

Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Chicago. Male Wistar strain rats (Harlan Sprague Dawley, Indianapolis, Ind.), weighing between 150 and 300 g, were used in this study. Animals were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with a 12-h light/12-h dark cycle. Rats were allowed free access to water, standard laboratory rat chow (Harlan-Teklad, Madison, Wis.) and kaolin (see below), placed in separated containers continuously available throughout the experiment.

Preparation and analysis of AGBE

Dried American ginseng berries (Wisconsin Ginseng Board Wausau, Wis.) were ground into fine powder, dispersed in 75% ethanol, sonicated and refluxed for 1 h at 50°C, as described previously [18]. The extraction procedure was repeated three times. The cooled mixture was filtered through Whatman No.1 paper (Maidstone, UK) and the filtrate was collected. The final residue was washed with ethanol and combined with the extract. The ethanol extract was dried under vacuum with a rotary evaporator R-205 (Buchi Labortechnik, Switzerland), dissolved in water, and partitioned with water-saturated butanol (three times). The butanol layer was combined, washed with water, evaporated under vacuum, and freeze-dried. The dried extracts were water-soluble and were used for the experiments.

HPLC analysis

HPLC was performed to confirm the ginsenoside profile of the AGBE using a Shimadzu liquid chromatography system (Kyoto, Japan) on a Phenomenex Prodigy C18, 5 μm, 150×3.2 mm analytical column (Phenomenex, Torrance, Calif.) [19]. Separation of ginsenosides was obtained at room temperature by gradient elution using water and acetonitrile as eluents. The UV detector range was 0.01 AUFS (absorbance unit full scale) and the absorbance was measured at 202 nm. Standards (ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) for HPLC analysis were obtained from Indofine Chemical Company (Somerville, N.J.). Ginsenoside Re (purity >98%; Indofine Chemical Company) was also tested for its effect on cisplatin-induced pica.

Kaolin preparation

Kaolin was prepared based on a previously described method [20]. Briefly, pharmacological grade kaolin (hydrated aluminum silicate; Fisher, Fair Lawn, N.J.) and acacia (Gum Arabic; Fisher, Fair Lawn, N.J.) were mixed at a ratio of 99:1. Distilled water was used to form a thick paste of this mixture. The paste was rolled and cut into pieces that resembled regular rat chow pellets. The pellets were dried at room temperature for 72 h.

Experimental protocol

Rats placed in individual cages were allowed access to both regular food and kaolin during the 3-day adaptation period prior to the study period (day 0).

Based on our earlier results, cisplatin at a dose 3 mg/kg IP was used to induce pica [20]. On day 0, all five groups of rats received two IP injections at 2 p.m. (pretreatment) and 2:30 p.m. (treatment). Group 1 animals ($n=4$) received normal saline and normal saline (NS + NS). Group 2 animals ($n=6$) received normal saline and cisplatin 3 mg/kg (NS + Cisp). Group 3 animals ($n=6$) received AGBE 50 mg/kg and cisplatin 3 mg/kg (AGBE 50 mg + Cisp). Group 4 animals ($n=6$) received AGBE 100 mg/kg and cisplatin 3 mg/kg (AGBE 100 mg + Cisp). Group 5 animals ($n=6$) received AGBE 150 mg/kg and cisplatin 3 mg/kg (AGBE 150 mg + Cisp). Additionally, two groups were pretreated with ginsenoside Re, followed by cisplatin administration (Re 2 mg + Cisp and Re 5 mg + Cisp; $n=5$ or 6 per group).

At 3 p.m. on each experimental day (five consecutive days), kaolin intake (g), food intake (g), and body weight (g) were measured. To measure kaolin and food intake, the remaining kaolin and food from the day prior was collected including that spilled outside the containers. The collected kaolin and food were dried for 72 h to obtain dry weight values to the nearest 0.1 g.

The animals did not demonstrate any adverse effects such as restlessness or respiratory distress following the injections.

Measurement of in vitro free radical scavenging ability of AGBE using ESR spectroscopy

The antioxidant activity of AGBE was evaluated on the basis of its scavenging effect on superoxide anion and hydroxyl radical using ESR spectroscopy. Hydrogen peroxide, ammonium iron (II) sulfate hexahydrate, xanthine, diethylenetriaminepentaacetic acid (DTPA), superoxide dismutase, and spin-trap 5,5-dimethyl *N*-oxide pyrroline (DMPO) were purchased from Sigma (St. Louis, Mo.), and xanthine oxidase (XOD) came from Roche Applied Science (Indianapolis, Ind.). The nitrone spin trap, 5-*tert*-butoxycarbonyl 5-methyl-1-pyrroline *N*-oxide (BMPO) was a gift from Prof. B. Kalyanaraman (Medical College of Wisconsin).

The ESR spin-trapping method was used to evaluate the superoxide and the hydroxyl radical scavenging activity of AGBE. Superoxide scavenging by AGBE was measured using the spin-trap BMPO [21]. Superoxide was produced by reaction of the xanthine/xanthine oxidase system and reacted with spin-trap BMPO. The formation of the BMPO-OOH adduct was detected using ESR spectroscopy. All the chemicals were dissolved in phosphate-buffered saline (PBS, 0.1 *M*, pH 7.4). ESR signal was recorded 5 min after 20 µl xanthine oxidase (0.25 U/ml, fresh made) was mixed with 20 µl xanthine (5 m*M*), 20 µl DTPA (0.5 m*M*), 20 µl BMPO (125 m*M*), 20 µl control (buffer) or different concentrations of AGBE.

Hydroxyl radical scavenging was measured using the spin-trap DMPO [22]. Hydroxyl radicals were generated by a Fenton reaction and reacted rapidly with spin-trap DMPO. The formation of the DMPO-OH adduct was detected using ESR spectroscopy. The ESR signal was recorded 2 min after the addition of 10 µl $FeSO_4$ (3 m*M*, fresh made) mixed with 10 µl DMPO (1 *M*), 10 µl H_2O_2 (0.5 m*M*), 50 µl PBS (pH 7.4) and 20 µl control (buffer) or different concentrations of AGBE.

Conventional ESR spectra were obtained with a Varian E-109 X-band spectrometer. ESR signals were recorded with 15 mW (for both DMPO-OH and BMPO-OOH) incident microwave and 100 kHz field modulation of 1G (for both DMPO-OH and BMPO-OOH). All measurements were performed at room temperature [22].

Statistical analysis

Data were analyzed using a two-way analysis of variance (ANOVA) with group and time as the two factors. Post-hoc comparisons between groups were performed using a Holm-Sidak test for multiple comparisons. Statistical significance was considered for P values < 0.05.

Results

Kaolin intake (pica) was measured in rats treated with cisplatin (3 mg/kg) that received either saline or AGBE pretreatment. Figure 1 demonstrates that AGBE pretreatment significantly attenuated kaolin intake induced by cisplatin. Cisplatin induced a significant increase in kaolin intake in the NS + Cisp group at 24, 48, 72, 96 and 120 h compared to 0 h ($P < 0.05$). Following pretreatment with AGBE, a dose-response effect in decreasing kaolin intake was observed. When rats were pretreated with AGBE 50 mg/kg, kaolin intake decreased significantly compared to the NS + Cisp group at 24, 48, 72 and 96 h ($P < 0.01$). Although there was a reduction compared to the NS + Cisp group, kaolin intake at 24 h (2.1 ± 0.3 g) was significantly greater than the corresponding baseline intake at 0 h (0.6 ± 0.09 g; $P < 0.01$). Pretreatment with AGBE 100 mg/kg significantly reduced kaolin intake from the NS + Cisp group at 24, 48, and 72, 96 h and also at 120 h ($P < 0.01$). Additionally the kaolin consumption at these time-points was not different from that at 0 h. This suggests that AGBE at 100 mg/kg attenuated pica for longer and to a greater magnitude compared to AGBE at 50 mg/kg. With an increase in the dose of AGBE to 150 mg/kg, kaolin intake was significantly reduced at 24 and 48 h ($P < 0.01$) compared to the NS + Cisp group. However, compared to its baseline kaolin intake at 0 h, there was a significant increase at 24 and 48 h. Thus with a pretreatment dose of 150 mg/kg AGBE, pica was not attenuated as effectively as at 50 or 100 mg/kg AGBE. The NS + NS group did not show any difference in

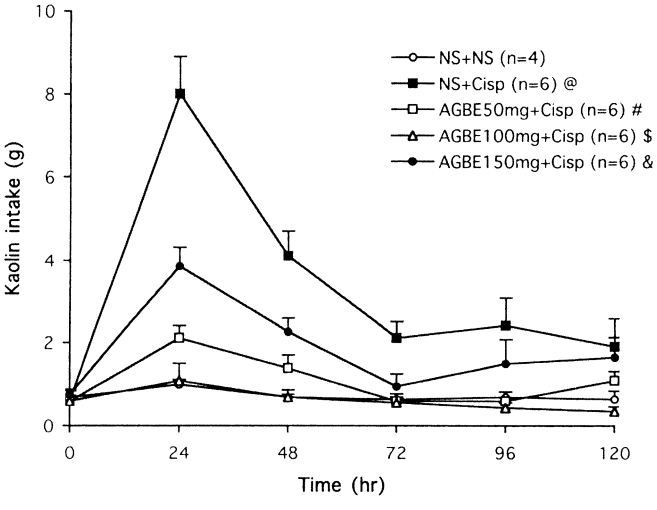

Fig. 1 Dose-related effects of pretreatment with AGBE (mg/kg) on kaolin intake (g) induced by cisplatin in rats. Increased kaolin intake induced by cisplatin was decreased with AGBE pretreatment. $^{@}P < 0.05$: 24, 48, 72, 96 and 120 h vs 0 h (baseline). $^{\#}P < 0.01$: different from NS + Cisp at 24, 48, 72 and 96 h, and from baseline at 24 h. $^{\$}P < 0.01$: different from NS + Cisp at 24, 48, 72, 96 and 120 h; no difference from baseline at 24, 48, 72, 96 or 120 h. $^{\&}P < 0.01$: different from NS + Cisp at 24 and 48 h, and from baseline at 24 and 48 h (*NS* normal saline, *Cisp* cisplatin 3 mg/kg)

kaolin intake during the experiment when compared to its baseline (0 h).

Figure 2 shows the effect of pretreatment with AGBE on food intake following cisplatin administration. Treatment with cisplatin in the NS + Cisp group resulted in a significant reduction in food intake at 24 h (57% of baseline) and 48 h (76% of baseline) compared to the NS + NS (control) group ($P < 0.01$). When pretreated with AGBE 50 mg/kg, food intake was significantly reduced at 24 h (76% of baseline) and 48 h (81% of baseline), compared to the NS + NS group ($P < 0.05$). When pretreated with AGBE 100 mg/kg, food intake decreased significantly only at 24 h (85% of baseline) compared to the NS + NS group ($P < 0.05$). Additionally, the magnitude of the reduction in food intake (reduced by 15%) in this group was significantly lower when compared to the NS + Cisp group (reduced by 43%; $P < 0.05$), suggesting that AGBE significantly attenuated the reduction in food intake induced by cisplatin at 24 and 48 h. However, when pretreated with AGBE 150 mg/kg, the cisplatin-induced reduction in food intake was worsened and significantly decreased compared to NS + NS group at 24, 48, 72 and 96 h ($P < 0.05$).

As shown in Fig. 3, there was a dose-related effect of pretreatment with ginsenoside Re on cisplatin-induced pica. Kaolin intake was not reduced by pretreatment with Re 2 mg/kg. In the Re 5 mg/kg + Cisp group, kaolin intake was significantly attenuated compared to the NS + Cisp group at 24 and 48 h ($P < 0.05$). Although there was a reduction compared to the NS + Cisp group, kaolin intake at 24 h (3.35 ± 0.97 g) was significantly greater than the corresponding baseline intake at 0 h (0.50 ± 0.13 g; $P < 0.05$).

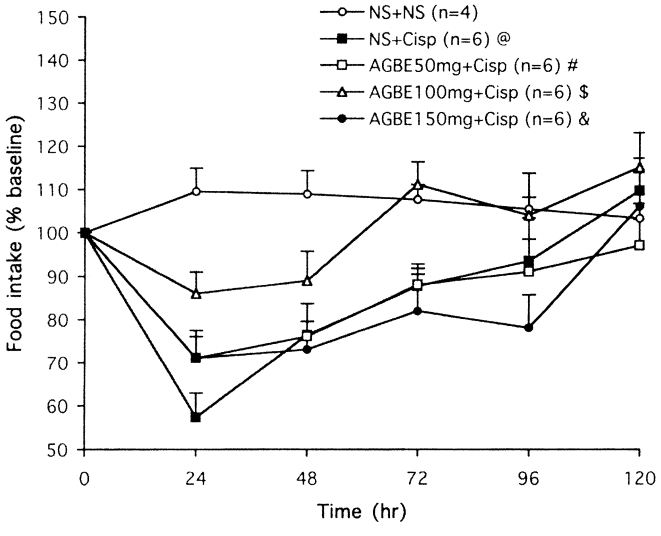

Fig. 2 Effects of pretreatment with AGBE (mg/kg) on reduced food intake induced by cisplatin in rats. The reduced food intake (expressed as percentage in relation to baseline) caused by cisplatin (3 mg/kg) was prevented by pretreatment with AGBE. $^{@}P < 0.01$: different from NS + NS (control) at 24 and 48 h. $^{\#}P < 0.05$: different from NS + NS at 24 and 48 h. $^{\$}P < 0.05$: different from NS + NS at 24 h, and from NS + Cisp at 24 h. $^{\&}P < 0.05$: different from NS + NS at 24, 48, 72 and 96 h (*NS* normal saline, *Cisp* cisplatin 3 mg/kg)

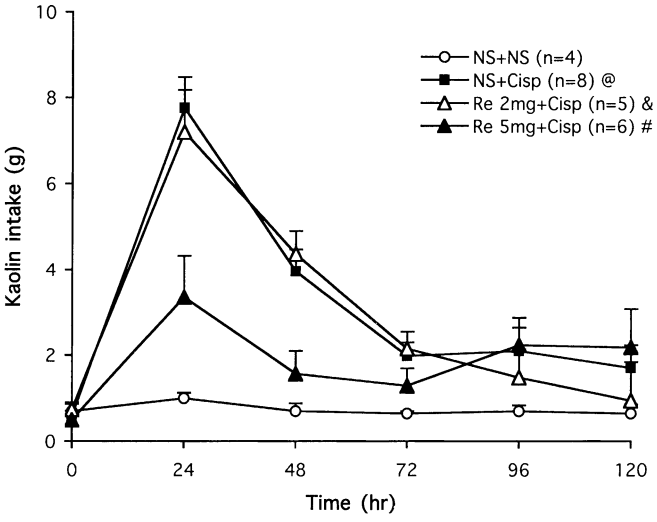

Fig. 3 Comparison of cisplatin-induced kaolin intake in the NS- and ginsenoside Re-pretreated groups. Ginsenoside Re (5 mg/kg) partly attenuated cisplatin-induced pica. $^{@}P < 0.05$: different from 0 h (baseline) at 24, 48, 72, 96 and 120 h. $^{\&}P < 0.05$ not different from NS + Cisp at 24, 48, 72, 96 or 120 h. $^{\#}P < 0.05$: different from NS + Cisp at 24 and 48 h (*NS* normal saline, *Cisp* cisplatin 3 mg/kg)

The in vitro radical scavenging activities of AGBE are shown in Fig. 4. Addition of AGBE to the xanthine/xanthine oxidase + BMPO system reduced the ESR signal of BMPO-OOH adduct dramatically (Fig. 4a). Efficient superoxide anion scavenging properties of AGBE were observed as a lowering of the ESR signal compared to that of the control. The same phenomenon was observed with the antioxidant enzyme superoxide dismutase (not shown). Figure 4b shows the typical 1:2:2:1 4 line ESR signal (with hyperfine splitting parameter $a^N = a^H = 14.9$ G) of DMPO-OH adducts formed by reaction of the spin-trap with the hydroxyl radical. Addition of AGBE appeared to reduce the ESR signal intensity of the DMPO-OH adduct. The superoxide scavenging ability of AGBE also appeared to be considerably more potent than the hydroxyl scavenging activity.

Discussion

In this study, we observed that extract from American ginseng berry attenuated kaolin intake (pica) and improved food intake in cisplatin-treated rats. We also demonstrated that ginsenoside Re, a major ginsenoside found in AGBE, attenuated cisplatin-induced pica, suggesting that Re may partly contribute to AGBE's effect. Additionally, we confirmed the antioxidant activity of AGBE, evaluated in vitro, which may be one of the mechanisms by which AGBE attenuates cisplatin-induced toxicity.

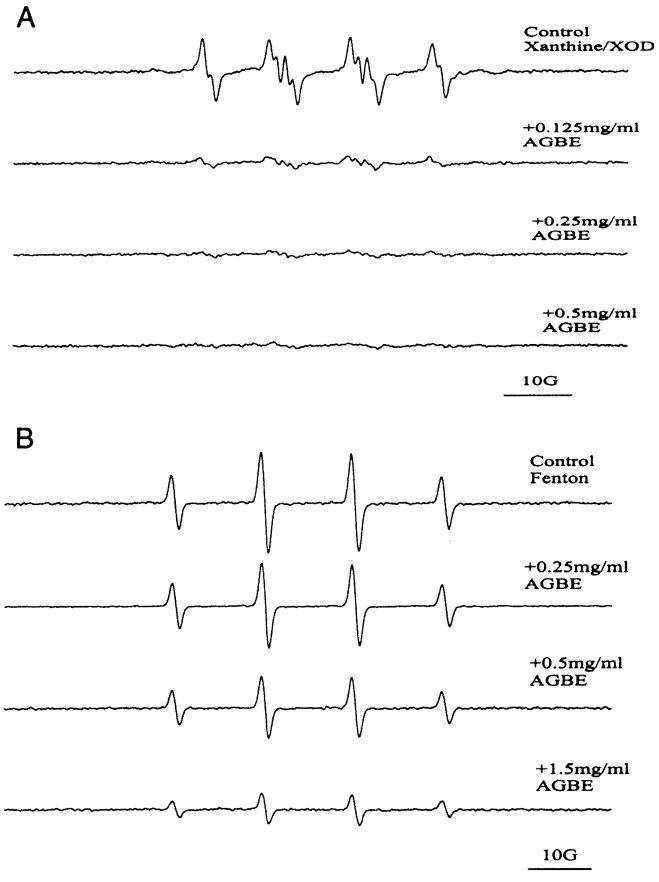

Fig. 4 **a** Effects of different concentrations of AGBE on the ESR spectra of BMPO/OOH and superoxide anion scavenging activity of AGBE. Superoxide was produced by reaction of using xanthine/xanthine oxidase (*XOD*) and reacted with the spin trap BMPO. **b** ESR spectrum of the DMPO/OH and hydroxyl radical scavenging activity of AGBE at different concentrations. Hydroxyl radicals were generated using a Fenton reaction between H_2O_2 and $FeSO_4$

Several animal models have been used for evaluating emetic and antiemetic compounds including dogs, ferrets, house musk shrew, and rats. Each model is associated with some limiting factor such as cost, ease of handling the animal, absence of vomiting center, and inability to vomit, respectively [13, 23– 26]. We used the rat model of simulated emesis in which rodents respond to emetic stimuli by increasing intake of non-nutritive substances such as kaolin [11–13]. Rats lack the motor component of the emetic reflex arc and therefore are unable to produce emesis [27]. Although not by vomiting, the rat model responds consistently to a variety of emesis-producing stimuli, such as cisplatin, morphine, simulated motion sickness and radiation, with pica [13, 28–31]. The assumption that pica in rats is analogous to emesis is further validated by the observation that antiemetic drugs attenuate pica [14, 15].

Cisplatin and other chemotherapeutic drugs and radiotherapy have been postulated to produce emesis by causing oxidant injury to the rapidly dividing mucosal layer of the intestines, including serotonin-producing cells (enterochromaffin cells) [6, 7, 32–34]. The consequent release of serotonin mediates emesis through stimulation of vagal nerve endings in the upper gastrointestinal tract [27, 35]. In humans cisplatin-induced emesis is manifested in two phases; an acute phase that lasts for approximately 24 h and a delayed phase that lasts for about 120 h [36, 37]. It has been observed clinically that the early phase is responsive to serotonin receptor antagonists ($5HT_3$ antagonists) and the delayed phase is responsive to neurokinin receptor antagonists [37]. Thus it appears that serotonin predominantly mediates the early emetic phase following cisplatin treatment while substance P mediates the delayed emesis. Substance P may be released from the injured intestinal epithelium along with serotonin or in the brainstem, where it is released by vagal afferent stimulation [27]. Since the initiating event that causes emesis appears to be oxidant damage to the intestinal mucosa, preventing mucosal injury by antioxidants could prevent cisplatin-induced emesis.

The present study tested whether pretreatment with an antioxidant herb, American ginseng berry, would attenuate cisplatin-induced pica. With the lower dose of AGBE (50 mg/kg) pica was significantly attenuated, but there was no improvement in food intake. The most effective dose was AGBE 100 mg/kg, which significantly eliminated pica from 24 to 120 h, and also significantly improved food intake. When the AGBE dose was further increased, however, the protective effect was reversed. Thus the group pretreated with AGBE 150 mg/kg demonstrated pica, although attenuated compared to the NS + Cisp group, at both 24 and 48 h, and demonstrated a prolonged reduction in food intake from 24 to 96 h. This suggests that doses higher than the therapeutic doses of AGBE could result in toxicity. Although the mechanism of this biphasic response is unclear, it is possible that at higher AGBE doses, the concentration of the prooxidant ginsenosides in the extract may sufficiently increase to induce oxidant toxicity [38, 39].

The antioxidant activity of AGBE may mediate the reduction in pica induced by cisplatin. It was demonstrated that American ginseng berry extract possesses significant antioxidant activity in both in vivo and in vitro evaluations, similar to American ginseng root and other antioxidant herbs [18, 40, 41]. Data from our study demonstrated a concentration-response effect of AGBE on the scavenging of superoxide and hydroxyl radicals. The dramatic superoxide anion scavenging effect of AGBE confirms its antioxidant activity and could contribute to the attenuation of cisplatin-induced pica [42, 43]. Apart from being a potent antioxidant, ginseng is also known to antagonize $5HT_3$ receptors and neurokinin receptors [44–46]. Thus ginseng may not only attenuate the oxidant gut injury but could also exert its anti-pica effects by other mechanisms.

In addition to testing the effectiveness of AGBE, we also evaluated the anti-pica effect of a major constituent of the extract, ginsenoside Re. The antioxidant property of ginsenosides has been demonstrated previously [38,

47]. In the current study the rats pretreated with Re (5 mg/kg) experienced a partial attenuation of cisplatin-induced pica. This suggests that Re may contribute to the AGBE-mediated reduction in pica and that AGBE may mediate its action, at least partly, by antioxidant mechanisms.

Antioxidants, including vitamins E and C, have been tested for their effectiveness in reducing oxidant-induced emesis or pica [5, 20]. We have previously demonstrated that pretreatment with 3 mg/kg of *Scutellaria baicalensis* extract (SbE), an antioxidant herb composed of flavonoid compounds, attenuates cisplatin-induced pica in rats [20]. We observed, however, that cisplatin-induced reduction in food intake does not improve significantly with SbE at that dose. In the present study AGBE 100 mg/kg completely reversed pica and significantly improved food intake. The reason for the differential effects of the two herbal antioxidants on kaolin intake and food intake is not clear, but may be related to the differences in composition and the pharmacology of the two botanicals. Alternatively, the SbE dose of 3 mg/kg may not have been high enough to improve food intake.

Antioxidants such as AGBE may attenuate chemotherapy-induced toxicity, but they could also compromise the tumoricidal activity of chemotherapeutic drugs [48]. It is important therefore to demonstrate that AGBE-induced attenuation of cisplatin-induced emesis is not accompanied with a reduced pharmacological activity of the chemotherapeutic agent. Although there could be several potential benefits to using ginseng with cisplatin [49–52], such use cannot be considered prior to demonstrating ginseng's non-interference with the pharmacokinetics and pharmacodynamics of chemotherapeutic agents.

We conclude that AGBE-induced reduction in pica and a simultaneous improvement in food intake in a cisplatin-treated rodent model suggest an antinausea/antiemetic action of AGBE. We propose that American ginseng berry extract possesses potential for treating chemotherapy-induced nausea and vomiting. The antiemetic efficacy of American ginseng, however, needs to be confirmed in other animal models of emesis prior to clinical testing.

Acknowledgements This work was supported in part by NIH grants P30 CA14599, R01 CA79042, AT00563 and AT002176.

References

1. Schnell FM (2003) Chemotherapy-induced nausea and vomiting: the importance of acute antiemetic control. Oncologist 8:187–198
2. Spitzer TR, Friedman CJ, Bushnell W, Frankel SR, Raschko J (2000) Double-blind, randomized, parallel-group study on the efficacy and safety of oral granisetron and oral ondansetron in the prophylaxis of nausea and vomiting in patients receiving hyperfractionated total body irradiation. Bone Marrow Transplant 26:203–210
3. The Italian Group for Antiemetic Research in Radiotherapy (1999) Radiation-induced emesis: a prospective observational multicenter Italian trial: the Italian Group for Antiemetic Research in Radiotherapy. Int J Radiat Oncol Biol Phys 44:619–625
4. Matsuki N (1996) Mechanisms of cytotoxic drug-induced emesis and its prevention. Yakugaku Zasshi 116:710–718
5. Torii Y, Mutoh M, Saito H, Matsuki N (1993) Involvement of free radicals in cisplatin-induced emesis in Suncus murinus. Eur J Pharmacol 248:131–135
6. Scarantino CW, Ornitz RD, Hoffman LG, Anderson RF Jr (1994) On the mechanism of radiation-induced emesis: the role of serotonin. Int J Radiat Oncol Biol Phys 30:825–830
7. Cubeddu LX (1992) Mechanisms by which cancer chemotherapeutic drugs induce emesis. Semin Oncol 19:2–13
8. Fukui H, Yamamoto M, Ando T, Sasaki S, Sato S (1993) Increase in serotonin levels in the dog ileum and blood by cisplatin as measured by microdialysis. Neuropharmacology 32:959–968
9. Simpson K, Spencer CM, McClellan KJ (2000) Tropisetron: an update of its use in the prevention of chemotherapy-induced nausea and vomiting. Drugs 59:1297–1315
10. Nitta Y, Nishibori M, Iwagaki H, Yoshino T, Mori S, Sawada K, Nakaya N, Saeki K, Tanaka N (2001) Changes in serotonin dynamics in the gastrointestinal tract of colon-26 tumour-bearing mice: effects of cisplatin treatment. Naunyn Schmiedebergs Arch Pharmacol 364:329–334
11. Mitchell D, Wells C, Hoch N, Lind K, Woods SC, Mitchell LK (1976) Poison induced pica in rats. Physiol Behav 17:691–697
12. Mitchell D, Krusemark ML, Hafner D (1977) Pica: a species relevant behavioral assay of motion sickness in the rat. Physiol Behav 18:125–130
13. Takeda N, Hasegawa S, Morita M, Matsunaga T (1993) Pica in rats is analogous to emesis: an animal model in emesis research. Pharmacol Biochem Behav 45:817–821
14. Rudd JA, Yamamoto K, Yamatodani A, Takeda N (2002) Differential action of ondansetron and dexamethasone to modify cisplatin-induced acute and delayed kaolin consumption ("pica") in rats. Eur J Pharmacol 454:47–52
15. Takeda N, Hasegawa S, Morita M, Horii A, Uno A, Yamatodani A, Matsunaga T (1995) Neuropharmacological mechanisms of emesis. II. Effects of antiemetic drugs on cisplatin-induced pica in rats. Methods Find Exp Clin Pharmacol 17:647–652
16. Ozaki A, Sukamoto T (1999) Improvement of cisplatin-induced emesis and delayed gastric emptying by KB-R6933, a novel 5-HT3 receptor antagonist. Gen Pharmacol 33:283–288
17. Foss JF, Yuan CS, Roizen MF, Goldberg LI (1998) Prevention of apomorphine- or cisplatin-induced emesis in the dog by a combination of methylnaltrexone and morphine. Cancer Chemother Pharmacol 42:287–291
18. Shao ZH, Xie JT, Van den Hoek TL, Mehendale S, Aung H, Li CQ, Qin Y, Schumacker PT, Becker LB, Yuan CS (2004) Antioxidant effects of American ginseng berry extract in cardiomyocytes exposed to acute oxidant stress. Biochim Biophys Acta 1670:165–171
19. Xie JT, Mehendale SR, Wang A, Han AH, Wu JA, Osinski J, Yuan CS (2004) American ginseng leaf: ginsenoside analysis and hypoglycemic activity. Pharmacol Res 49:113–117
20. Aung HH, Dey L, Mehendale S, Xie JT, Wu JA, Yuan CS (2003) Scutellaria baicalensis extract decreases cisplatin-induced pica in rats. Cancer Chemother Pharmacol 52:453–458
21. Zhao H, Joseph J, Zhang H, Karoui H, Kalyanaraman B (2001) Synthesis and biochemical applications of a solid cyclic nitrone spin trap: a relatively superior trap for detecting superoxide anions and glutathiyl radicals. Free Radic Biol Med 31:599–606
22. Yasuko N, Masahiro K, Akitane M, Packer L (1999) Automated electron spin resonance free radical detector assays for antioxidant activity in natural extracts. Methods Enzymol 299:28–34
23. Ueno S, Matsuki N, Saito H (1987) Suncus murinus: a new experimental model in emesis research. Life Sci 41:513–518

24. Milano S, Blower P, Romain D, Grelot L (1995) The piglet as a suitable animal model for studying the delayed phase of cisplatin-induced emesis. J Pharmacol Exp Ther 274:951–961
25. King GL, Rabin BM, Weatherspoon JK (1999) 5-HT3 receptor antagonists ameliorate emesis in the ferret evoked by neutron or proton radiation. Aviat Space Environ Med 70:485–492
26. King GL (1990) Animal models in the study of vomiting. Can J Physiol Pharmacol 68:260–268
27. Andrews PL, Sanger GJ (2002) Abdominal vagal afferent neurones: an important target for the treatment of gastrointestinal dysfunction. Curr Opin Pharmacol 2:650–656
28. Morita M, Takeda N, Kubo T, Matsunaga T (1988) Pica as an index of motion sickness in rats. ORL J Otorhinolaryngol Relat Spec 50:188–192
29. Mitchell D, Laycock JD, Stephens WF (1977) Motion sickness-induced pica in the rat. Am J Clin Nutr 30:147–150
30. Aung HH, Mehendale SR, Xie JT, Moss J, Yuan CS (2004) Methylnaltrexone prevents morphine-induced kaolin intake in the rat. Life Sci 74:2685–2691
31. Yamamoto K, Takeda N, Yamatodani A (2002) Establishment of an animal model for radiation-induced vomiting in rats using pica. J Radiat Res (Tokyo) 43:135–141
32. Stankiewicz A, Skrzydlewska E, Makiela AM (2002) Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats. Drug Metabol Drug Interact 19:67–82
33. Duncan M, Grant G (2003) Oral and intestinal mucositis—causes and possible treatments. Aliment Pharmacol Ther 18(9):853–874
34. Minami M, Ogawa T, Endo T, Hamaue N, Hirafuji M, Yoshioka M, Blower PR, Andrews PL (1997) Cyclophosphamide increases 5-hydroxytryptamine release from the isolated ileum of the rat. Res Commun Mol Pathol Pharmacol 97:13–24
35. Minami M, Endo T, Hirafuji M, Hamaue N, Liu Y, Hiroshige T, Nemoto M, Saito H, Yoshioka M (2003) Pharmacological aspects of anticancer drug-induced emesis with emphasis on serotonin release and vagal nerve activity. Pharmacol Ther 99:149–165
36. Hesketh PJ, Van Belle S, Aapro M, Tattersall FD, Naylor RJ, Hargreaves R, Carides AD, Evans JK, Horgan KJ (2003) Differential involvement of neurotransmitters through the time course of cisplatin-induced emesis as revealed by therapy with specific receptor antagonists. Eur J Cancer 39:1074–1080
37. Kris MG, Roila F, De Mulder PH, Marty M (1998) Delayed emesis following anticancer chemotherapy. Support Care Cancer 6:228–232
38. Liu ZQ, Luo XY, Liu GZ, Chen YP, Wang ZC, Sun YX (2003) In vitro study of the relationship between the structure of ginsenoside and its antioxidative or prooxidative activity in free radical induced hemolysis of human erythrocytes. J Agric Food Chem 51:2555–2558
39. Zheng W, Wang SY (2003) Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. J Agric Food Chem 51:502–509
40. Kitts D, Hu C (2000) Efficacy and safety of ginseng. Public Health Nutr 3:473–485
41. Shao ZH, Van den Hoek TL, Li CQ, Schumacker PT, Becker LB, Chan KC, Qin Y, Yin JJ, Yuan CS (2004) Synergistic effect of Scutellaria baicalensis and grape seed proanthocyanidins on scavenging reactive oxygen species in vitro. Am J Chin Med 32:89–95
42. Dehne N, Lautermann J, Petrat F, Rauen U, de Groot H (2001) Cisplatin ototoxicity: involvement of iron and enhanced formation of superoxide anion radicals. Toxicol Appl Pharmacol 174:27–34
43. Davis CA, Nick HS, Agarwal A (2001) Manganese superoxide dismutase attenuates cisplatin-induced renal injury: importance of superoxide. J Am Soc Nephrol 12:2683–2690
44. Yoon SR, Nah JJ, Shin YH, Kim SK, Nam KY, Choi HS, Nah SY (1998) Ginsenosides induce differential antinociception and inhibit substance P induced-nociceptive response in mice. Life Sci 62:PL319–PL325
45. Sampson JH, Phillipson JD, Bowery NG, O'Neill MJ, Houston JG, Lewis JA (2000) Ethnomedicinally selected plants as sources of potential analgesic compounds: indication of in vitro biological activity in receptor binding assays. Phytother Res 14:24–29
46. Min KT, Koo BN, Kang JW, Bai SJ, Ko SR, Cho ZH (2003) Effect of ginseng saponins on the recombinant serotonin type 3A receptor expressed in xenopus oocytes: implication of possible application as an antiemetic. J Altern Complement Med 9:505–510
47. Deng HL, Zhang JT (1991) Anti-lipid peroxidative effect of ginsenoside Rb1 and Rg1. Chin Med J (Engl) 104:395–398
48. Lamson DW, Brignall MS (1999) Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. Altern Med Rev 4:304–329
49. Lee SJ, Sung JH, Moon CK, Lee BH (1999) Antitumor activity of a novel ginseng saponin metabolite in human pulmonary adenocarcinoma cells resistant to cisplatin. Cancer Lett 144:39–43
50. Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tonooka S, Samukawa K, Azuma I (1995) Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)- and 20(S)-ginsenoside-Rg3, of red ginseng. Biol Pharm Bull 18:1197–1202
51. Yokozawa T, Liu ZW (2000) The role of ginsenoside-Rd in cisplatin-induced acute renal failure. Renal Fail 22:115–127
52. Rybak LP, Kelly T (2003) Ototoxicity: bioprotective mechanisms. Curr Opin Otolaryngol Head Neck Surg 11:328–333