Effect of Ginseng Saponins on a Rat Visceral Hypersensitivity Model

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The 5-hydroxytryptamine$_{3A}$ (5-HT$_3$) receptor is closely related with irritable bowel syndrome (IBS) in enteric nervous systems. We previously demonstrated that ginseng total saponins (GTS, also called ginsenosides), the active ingredients of *Panax ginseng*, inhibit the activity of 5-HT$_{3A}$ receptor channels expressed in *Xenopus laevis* oocytes. Here, we further investigated whether the *in vitro* inhibitory effect of ginsenosides on 5-HT$_{3A}$ receptor channel activity is coupled to *in vivo* attenuation of IBS. A rat model of IBS was induced by colorectal distention (CRD) and intracolonic infusion of 0.6% acetic acid (CRD-acetic acid), and visceral hypersensitivity was assessed by counting the contractions in the external oblique muscles of conscious rats during the 10 min distention period. We found that oral administration of GTS significantly and dose-dependently inhibited CRD-acetic acid-induced visceral hypersensitivity. The EC$_{50}$ was 5.5±4.7 mg/kg (95% confidence intervals: 1.2—15.7) and the inhibitory effect of GTS against visceral hypersensitivity persisted for 4 h. When we compared the effects of protopanaxadiol (PD) ginsenosides and protopanaxatriol (PT) ginsenosides against CRD-acetic acid-induced visceral hypersensitivity, we found that PT but not PD ginsenosides significantly attenuated the CRD-acetic acid-induced visceral hypersensitivity. These results indicate that PT ginsenosides of *Panax ginseng* might be the main active components for the attenuation of experimentally CRD-acetic acid-induced visceral hypersensitivity, and may be clinically relevant for the future treatment of IBS.

Key words ginsenoside saponins; 5-hydroxytryptamine$_{3A}$ receptor; irritable bowel syndrome

One of the most common gastrointestinal disorders without direct bacterial or viral infection is irritable bowel syndrome (IBS), which is strongly associated with stress. The most common IBS symptoms are abnormal visceral discomfort, pain and diarrhea. Although the precise mechanism of IBS has not been fully elucidated, some symptoms of IBS are thought to involve the 5-hydroxytryptamine receptor$_3$ (5-HT$_3$). Zacopride, a 5-HT$_3$-receptor antagonist, has been used to effectively treat diarrhea-predominant IBS patients with abdominal pain and bowel discomfort.[1] However, other 5-HT$_3$ receptor antagonists have demonstrated a wide heterogeneity of potency and efficacy against visceral pain; granisetron reduces rectal sensitivity in IBS patients, but ondansetron does not.[2,3] These and other findings seem to indicate that the 5-HT$_3$ receptors are associated with the nociceptive processes of visceral pain in both humans and animals,[4] but the precise mechanisms by which 5-HT$_3$-receptor antagonists inhibit the visceral pain remain unclear. Researchers are currently seeking to identify new 5-HT$_3$-receptor antagonists capable of blocking abnormal visceral perception without deleterious side effects.

Ginseng, the root of *Panax ginseng* C. A. MEYER, is well known for restoring and promoting human health. In traditional medicine, ginseng has been used as an antidote for stress and to alleviate disorders such as anorexia, dyspepsia, pain and vomiting.[5] The main molecular components responsible for the actions of *Panax* ginseng are the ginsenosides, which are also known as ginseng saponins. Approximately 30 different ginsenoside forms have been isolated and identified from the root of *Panax* ginseng. These molecules have a four-ring, steroid-like structure bearing sugar moieties, and can be classified into protopanaxadiol (PD) or protopanaxatriol (PT) ginsenosides according to the position of the sugar moieties at carbon-3 or -6 (Fig. 1).[6] Ginsenosides regulate several types of ligand-gated ion channel activity, and differential regulation is seen by the PT and PD forms. In cells expressing nicotinic acetylcholine receptors, such as bovine chromaffin cells, the PT ginsenosides, especially ginsenosides Rf and Rg$_2$, potently inhibit acetylcholine-stimulated Na^+ influx.[7] Choi *et al.* (2002) and Sala *et al.* (2002) showed that PT ginsenosides potently inhibited acetylcholine-induced inward currents in *Xenopus* oocytes expressing several subtypes of neuronal and muscle-type nicotinic acetylcholine receptors.[8,9] Lee *et al.* (2004) showed that PT ginsenosides and the PT ginsenoside metabolite, M4, inhibited 5-HT-mediated inward currents in *Xenopus* oocytes expressing 5-HT$_{3A}$ receptors to a greater degree than did PD ginsenosides and the PD ginsenoside metabolite, Compound K.[10] Since 5-HT$_{3A}$ receptors exist in enteric nervous systems and are involved in IBS, the observation that ginsenosides can block 5-HT$_{3A}$ receptor channel activity suggests that ginsenosides might alleviate IBS. However, no previous work has examined whether the regulation of 5-HT$_{3A}$ receptor channel activity by ginsenosides is coupled to attenuation of IBS.

Here, we investigated whether the inhibitory effects of ginsenosides on 5-HT$_{3A}$ receptor channel activity are coupled to the alleviation of visceral hypersensitivity *in vivo*. We induced experimental visceral hypersensitivity by colorectal distention (CRD) and intracolonic infusion of 0.6% acetic acid (CRD-acetic acid) in rats,[11] and examined the effect of ginsenosides (GTS, PT or PD) on CRD-acetic acid-induced visceral hypersensitivity as compared to the effect of zacopride, a known 5-HT$_3$ receptor antagonist. We found that ginsenosides dose-dependently reduced CRD-acetic acid-induced visceral hypersensitivity, with the PT ginsenosides showing a more significant effect than the PD ginsenosides.

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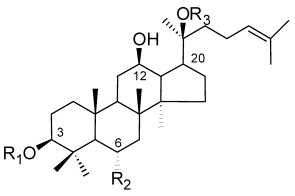

Ginsenosides	R_1	R_2	R_3
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc
Rb_2	-Glc_2-Glc	-H	-Glu_6-Ara(pyr)
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)
Rd	-Glc_2-Glc	-H	-Glc
Re	-H	-O-Glc_2-Rha	-Glc
Rf	-H	-O-Glc_2-Glc	-H
Rg_1	-H	-O-Glc	-Glc
Rg_2	-H	-O-Glc_2-Rha	-H
Rg_3	-Glc_2-Glc	-H	-H

Fig. 1. Structures of the Nine Representative Ginsenosides, Which Differ at Three Side Chains Attached the Common Steroid Ring

Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates. These molecules have a four-ring, steroid-like structure bearing sugar moieties, and can be classified into protopanaxadiol (PD) or protopanaxatriol (PT) ginsenosides according to the position of the sugar moieties at carbon-3 or -6.

MATERIALS AND METHODS

Materials Ginseng total saponins (GTS), PD and PT ginsenosides compounds, isolated according to the method of Tanaka *et al.* (1966),[12] were kindly provided by the Korea Ginseng and Tobacco Research Institute (Taejon, Korea). Figure 1 shows the structures of the representative ginsenosides. Ginsenosides have a four-ring, steroid-like structure bearing sugar moieties, and can be classified into protopanaxadiol (PD) or protopanaxatriol (PT) ginsenosides according to the position of the sugar moieties at carbon-3 or -6. The stock GTS solution was diluted with saline before use. Other chemical agents were obtained from Sigma (St. Louis, MO, U.S.A.). The ginsenosides were administered orally at doses ranging from 0.1 to 1000 mg/kg (GTS) or 100 mg/kg (PD and PT). Zacopride (4-amino-*N*-(1-azabicyclo[2.2.2.]oct-3-yl)-5-chloro-2-methobenza-mide) was administered subcutaneously (s.c.) at doses ranging from 0.1 to 300 μg/kg. All drugs were dissolved in saline, adjusted to pH 7.4, and administered in a volume of 1 ml/kg body weight.

Induction of Colorectal Distention (CRD)-Acetic Acid-Induced Hypersensitivity in Rats Male Sprague–Dawley rats (Orient, Korea) weighing 300—350 g were housed communally at 22±2 °C and given free access to food and water. The procedures for the maintenance and use of experimental animals were carried out in accordance with the guidelines of the International Association for the Study of Pain. CRD-acetic acid-induced visceral hypersensitivity was triggered according to the method of Langlois *et al.* (1996).[11] Briefly, each animal was fasted overnight, placed in a transparent plastic cage lined with sawdust, and allowed 60 min to acclimatize. The visceral stimulus employed in all experiments was distension of the descending colon by inflation of a 5-cm-long latex balloon inserted anally and kept in place by taping the polyethylene tube holding the balloon to the base of the tail such that the tip of the balloon remained 10 cm from the anal verge. In all experiments, the pressure distension was kept constant, as monitored with a pressure transducer (Bioblock, Illkirch, France). After insertion of the inflated balloon, 1.5 ml of 0.6% acetic acid was infused intracolonically through a small catheter mounted along the balloon assembly. After 1 h, a first period of 30 mmHg distension was applied for 10 min (control period).[11] This distension period was followed by oral administration of vehicle saline, GTS, PD or PT ginsenosides, or s.c. administration of zacopride. After 20 min, a second period of distension (30 mmHg for 10 min) was again applied (treatment period). CRD-acetic acid-induced visceral hypersensitivity was scored by visual countings of abdominal contractions over the two 10-min distension periods.[11]

Data Analysis The results are expressed as means± S.E.M. Statistically significant differences between the control and treatment groups were assessed using the Wilcoxon test. Differences were considered statistically significant at $p<0.05$. The effects of GTS, PD, PT ginsenosides and the 5-HT_3 receptor antagonist against visceral hypersensitivity were expressed by the following equation: % anti-hypersensitivity=100×[1−(AC after treatment/AC before treatment)] (AC=cumulative abdominal contraction). The ED_{50} was calculated using the method of Litchfield and Wilcoxon.[13]

RESULTS AND DISCUSSION

5-HT_{3A} receptor is not only involved in central nervous system control of vomiting and nausea, but is also related with IBS in the peripheral nervous system. We have demonstrated that the inhibitory effect of ginsenosides, the active ingredient of *Panax* ginseng, on 5-HT_{3A} receptor channel activity is coupled to its anti-vomiting and anti-nausea effects.[14] Here, we further investigated whether the inhibitory effect of ginsenosides on 5-HT_{3A} receptor channel activity is also coupled to attenuation of IBS in the peripheral nervous system. We generated an *in vivo* CRD-acetic acid-induced visceral hypersensitivity model in rats and examined the effect of GTS on this model.[11] We first tested reproducibility of our procedure in the absence of drug treatment. We observed a small number of abdominal contractions after intracolonic administration of saline followed by distension at 30 mmHg for 10 min (8.1±2.4 abdominal contractions during the distension period). In contrast, rats receiving intracolonic administration of 0.6% acetic acid followed 1 h later by distension showed a significant increase in abdominal contractions during the 10 min distension period (197.8±8.3; $p<0.01$ *vs.* the saline treatment group). This response was stable and reproducible, and a similar hypersensitivity was seen during a second distension applied 20 min later (203.9±5.3 abdominal contractions; data not shown).

We next tested the effect of GTS (0.1 to 300 mg/kg) against our experimentally induced visceral hypersensitivity. We triggered a first period of CRD-acetic acid-induced visceral hypersensitivity and measured abdominal contractions over the 10 min distension. We then administered the indicated doses of zacopride or GTS, waited 20 min, and then measured the abdominal contractions during a second 10 min period of CRD-acetic acid-induced visceral hypersensitivity. As shown in Fig. 2, GTS treatment significantly and dose-dependently inhibited the abdominal contractions, showing a slightly bell-shaped dose-response curve that fell off at the

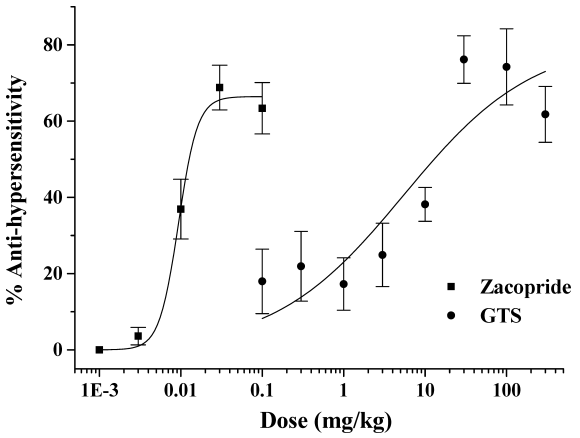

Fig. 2. Dose-Response Relationships of Anti-hypersensitivity Effects of GTS (○) or Zacopride (●) in Rats with CRD-Acetic Acid-Induced Visceral Hypersensitivity

CRD-acetic acid induced visceral hypersensitivity was induced and the % anti-hypersensitivity effects of GTS and zacopride were determined as described in Materials and Methods. Data are expressed as means±S.E.M. (n=11—12/dose).

highest concentration of GTS. The % anti-hypersensitivity induced by GTS treatment was 17.9±8.4, 21.9±9.1, 17.2±6.8, 24.9±8.3, 38.1±4.4, 76.1±6.2, 74.2±9.9 and 61.78±7.3% at doses of 0.1, 0.3, 1, 3, 10, 30, 100 and 300 mg/kg, respectively. The maximal saturated anti-hypersensitivity effects were observed at a dose of 30 mg/kg GTS, and these effects slightly decreased at a dose of 300 mg/kg GTS (Fig. 2). The EC_{50} for this response was 5.5±4.7 mg/kg (95% confidence intervals: 1.2—15.7). Zacopride, a known 5-HT_3 receptor antagonist, also significantly and dose-dependently inhibited abdominal contractions under these conditions. The % anti-hypersensitivity induced by zacopride was 0.0, 3.6±2.3, 36.9±7.8, 68.8±5.86, 63.4±6.7 and 36.5±7.6% at doses of 0.001, 0.003, 0.01, 0.03, 0.1 and 0.3 mg/kg, respectively. Zacopride showed saturation of the anti-hypersensitivity effects at 0.03 mg/kg, and yielded an EC_{50} value of 9.3±0.7 μg/kg (95% confidence intervals: 3.7—19.7) (Fig. 2), which is consistent with previously reported EC_{50} values for this drug.[11]

We then investigated the time-dependent effects of GTS or zacopride on CRD-acetic acid-induced visceral hypersensitivity. We measured abdominal contractions during a first 10 min period of CRD-acetic acid-induced visceral hypersensitivity, administered 0.1 mg/kg zacopride or 100 mg/kg GTS, and then measured a second 10 min period of CRD-acetic acid-induced visceral hypersensitivity triggered at various time points after drug treatment. At 5, 10, 20, 60, 120, 240 or 480 min after drug treatment, the % anti-hypersensitivity values were 3.7±4.2, 8.9±5.3, 4.8±4.7, 12.5±3.9, 10.8±9.1, 13.9±5.8 and 15.9±9.7%, respectively, in the saline-treated control group, 34.4±3.4, 65.4±2.7, 68.8±5.9, 60.2±3.2, 55.4±5.8, 19.1±8.2 and 8.9±4.7%, respectively, in the zacopride-treated group, and 30.5±2.6, 62.7±4.9, 76.1±6.2, 75.5±5.0, 71.1±2.8, 59.7±8 and 19.9±4.8%, respectively, in the GTS-treated group (n=12—13, each group). These findings indicate that the maximal anti-hypersensitivity effects of zacopride and GTS were seen at 20 min, while GTS but not zacopride maintained a significant anti-hypersensitivity effect after 240 min (Fig. 3A). Over the entire 480 min period, the zacopride and GTS treatments produced statistically significant anti-hypersensitivity effects (44.6±9.0 and 56.5±8.5%, respectively) *versus* the control (10.1±6.1) (Fig. 3B; p<0.01, compared to the saline treatment group).

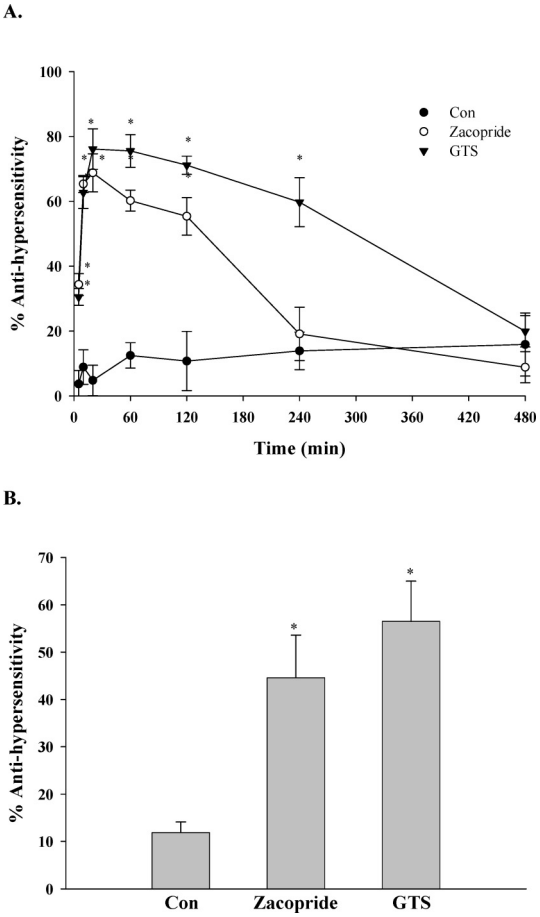

Fig. 3. Time–Response Relationships of the Anti-hypersensitivity Effects Produced by Zacopride and GTS

(A) Rats were pre-treated with zacopride for the indicated times (5, 10, 20, 60, 120, 240, 480 min) prior to the second induction of CRD-acetic acid-induced visceral pain, and the % anti-hypersensitivity effects of GTS and zacopride on CRD-acetic acid induced visceral hypersensitivity were determined as described in Materials and Methods. The GTS (▼) and zacopride (○) groups exhibited significantly improved % anti-hypersensitivities compared with the control group (●). *p<0.001, compared with the saline treated group (n=11—12/group). (B) Histograms summarizing the time-dependent anti-hypersensitivity effect of GTS and zacopride for 8 h on CRD-acetic acid induced visceral hypersensitivity. Data represent the mean±S.E.M. *p<0.01 compared with saline treatment alone (n=11—12/group).

Since we previously demonstrated that PT ginsenosides and their metabolite, M4, more potently inhibited 5-HT-mediated currents in *Xenopus* oocytes expressing 5-HT_{3A} receptor than did PD ginsenosides and their metabolite, compound K,[10] we next compared the anti-hypersensitivity effects of PD and PT ginsenosides in our CRD-acetic acid-induced visceral pain model. We measured abdominal contractions during the first 10 min period of CRD-acetic acid-induced visceral hypersensitivity, administered 100 mg/kg PD or PT ginsenosides, and then measured abdominal contractions induced by a second period of CRD-acetic acid-induced visceral hypersensitivity applied at various time points. After 5, 10, 20, 60, 120, 240 and 480 min, the % anti-hypersensitivity was 11.5±3.1, 4.3±2.7, 9.6±5.3, 7.8±3.9, 15.3±4.8, 22.9±7.1, and 12.0±6.8%, respectively, in the saline-treated control group, 5.8±4.8, 6.5±7.3, 5.0±6.9, 10.3±7.1,

26.5±5.9, 28.7±6.9 and 5.8±8.8%, respectively, in the PD ginsenoside-treated group, and 2.0±3.1, 27.8±9.1, 39.5±6.2, 47.7±8.8, 41.5±7.4, 32.8±5.4 and 3.2±2.9%, respectively, in the PT ginsenoside-treated group (n=12—13 each group). Thus, the PT ginsenosides showed large maximal anti-hypersensitivity effects after 60 min (47.7±8.8%), whereas the PD ginsenosides did not show significant anti-hypersensitivity effects after 60 min compared with control group (Fig. 4A). Over the total 480 min period, the control, PD- and PT ginsenoside-treated groups produced anti-hypersensitivity effects of 11.8±2.3, 12.6±3.9 and 27.7±6.9%, respectively, in CRD-acetic acid-induced visceral pain (Fig. 4B). Thus, PT but not PD ginsenosides showed significant average anti-hypersensitivity effects compared to the saline-treated control group (Fig. 4B) (p<0.01, compared to saline alone treatment).

Thus, we herein showed that oral administration of GTS and PT but not PD ginsenosides dose- and time-dependently attenuated CRD-acetic acid-induced visceral hypersensitivity in rats. These results indicate that the main components governing the attenuation of CRD-acetic acid-induced visceral hypersensitivity in GTS are derived from PT ginsenosides. It is interesting to speculate how GTS acts so quickly in this *in vivo* model, when ginsenosides were traditionally thought to be inefficiently absorbed following oral administration. However, recent reports have shown that some ginsenosides are rapidly absorbed by the intestines after oral administration and pass into the plasma within 20 min.[15,16] In contrast, other ginsenosides undergo metabolism by enteric microorganisms, which transform PD and PT ginsenosides into M4 and CK, respectively.[17] It seems unlikely that these ginsenoside metabolites could play a major role for the rapid attenuation of CRD-acetic acid-induced visceral hypersensitivity, since enteric microorganisms might require a longer time than that observed in present study for bio-transformation of orally administered ginsenosides. Therefore, two possibilities could explain the rapid ginsenoside-induced anti-hypersensitivity effect in observed in the present study: 1) the ginsenosides are rapidly absorbed into the plasma, where they act to quickly attenuate CRD-acetic acid-induced visceral hypersensitivity; or 2) the ginsenosides induce rapid attenuation of CRD-acetic acid-induced visceral hypersensitivity by direct interactions with the enteric nervous system, without plasma absorption. Future work will be required to examine these possibilities. Additional studies are also needed to examine why the PT ginsenosides showed lower anti-hypersensitivity effects than did GTS. This was unexpected, since active fractions of naturally active components usually exhibit more efficacy than the crude state. One possibility is that one or more active component(s) may be lost from the GTS during the fractionation process.

In terms of possible mechanisms by which GTS- and PT ginsenosides induce attenuation of CRD-acetic acid-induced visceral hypersensitivity, recent reports have shown that 5-HT_3 receptors are prevalent in the enteric nervous systems. Furthermore, abnormal activation of these receptors under various stressful conditions has been associated with IBS.[18] Our group and others have previously shown that GTS, PD ginsenosides, PT ginsenosides and their metabolites inhibit 5-HT-mediated inward currents in *Xenopus* oocytes expressing 5-HT_{3A} receptors.[10,19] Interestingly, the PT ginsenosides

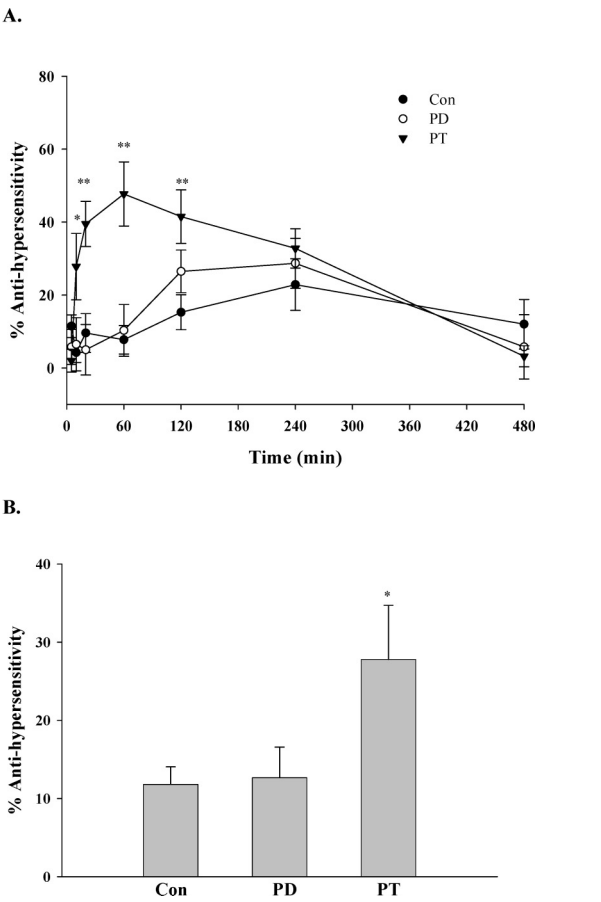

Fig. 4. Time–Response Relationships of Anti-hypersensitivity Effects Produced by PD or PT Ginsenosides

(A) CRD-acetic acid-induced visceral hypersensitivity was triggered by administration of 30 mmHg for 10 min and measured, rats were treated with 100 mg/kg PD or PT ginsenosides for 5, 10, 20, 60, 120, 240, 480 min, and then a second period of CRD-acetic acid induced visceral hypersensitivity was induced and measured. The maximal inhibition of CRD-acetic acid-induced visceral hypersensitivity by PT ginsenosides was 47.7±8.8% at 60 min. The responses in the PD (○)- and PT (▼)-treated groups were significantly different from that of the control saline-treated group, $*p$<0.05, $**p$<0.001 (n=10—12/group). (B) Histograms summarizing the time-dependent effect of PD and PT saponins for 8 h on CRD-acetic acid induced visceral anti-hypersensitivity. Data represent the mean±S.E.M. $*p$<0.01 compared with saline treatment alone (n=11—12/group).

and the M4, PT ginsenoside metabolite, were shown to more potently inhibit 5-HT-mediated inward currents than did the PD ginsenosides or the CK, PD ginsenoside metabolite. Thus, ginsenoside-induced inhibition of 5-HT_{3A} receptor channel activity in enteric nervous system might be one of underlying mechanisms against CRD-acetic acid-induced visceral hypersensitivity. However, other recent reports have shown that muscarinic acetylcholinergic receptors are also abundant in gastrointestinal systems, their antagonists attenuate IBS, and enhancements in their activity are followed by 5-HT_3 receptor activation, resulting in an increase of gastrointestinal motility and fluid secretion.[20] Furthermore, Saito *et al.* (1973) and Kaku *et al.* (1975) demonstrated that ginsenosides inhibit acetylcholine-mediated intestinal smooth muscle contractions.[21,22] Thus, it is possible that ginsenoside-mediated attenuation of CRD-acetic acid-induced visceral hypersensitivity could be linked to inhibition of acetylcholine-mediated intestinal smooth muscle contractions. The third and last possibility is that the stress-relieving effect of ginseng might mediate the palliative effect on CRD-

acetic acid induced visceral hypersensitivity, since ginseng is well known anti-stress agent and the main cause of IBS is persistent stress.

Finally, we compared our results to the effects of zacopride, a specific 5-HT_3 receptor antagonist. Zacopride effectively blocked CRD-acetic acid-induced visceral hypersensitivity. The EC_{50} values of zacopride were *ca.* 500-fold lower than those of GTS, but GTS treatment maintained its anti-hypersensitivity effect for more than 4 h, whereas zacopride-induced inhibition of CRD-acetic acid-induced visceral hypersensitivity dramatically decreased after 2 h (Fig. 3A). Thus, it seems that GTS has a longer duration than zacopride when considering the inhibition of CRD-acetic acid-induced visceral hypersensitivity. Moreover, since GTS is natural component and not a synthetic compound like zacopride, it did not show deleterious side effects at oral doses in the EC_{50} range.

In summary, we herein demonstrated that GTS dose- and time-dependently attenuates CRD-acetic acid-induced visceral hypersensitivity in rats, suggesting that ginsenosides might prove to be effective for use as antinociceptive agents in the clinical treatment of IBS.

Acknowledgements This research was supported by grants from the Neurobiology Research Program and the Bio-Food and Drug Research Center from the Korea Ministry of Science and Technology.

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