Acanthopanax senticosus Root Extract Exerts Dual Action on Mouse Ileal Smooth Muscle Function, Leading to Modulation of Gastrointestinal Motility

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Diarrhea is often caused by changes in lifestyle, stress, or side effects of drugs. Acanthopanax senticosus root extract (ASRE) has long been used as a functional food remedy with anti-fatigue, neuroprotective, and immunomodulatory activities. However, it is unclear whether ASRE has beneficial effects on gastrointestinal (GI) motility. Therefore, we first investigated whether ASRE directly affects contractile functions of the isolated mouse ileum, and then assessed its effects on GI transit of a charcoal meal in normal mice and a carbachol (CCh)-induced diarrhea mouse model. ASRE caused contraction of the isolated mouse ileum and the maximum contraction was approximately half of that induced by acetylcholine (ACh) administration. In the presence of atropine, this ASRE-induced contraction disappeared, while relaxation responses were observed. However, ASRE reduced potassium chloride- and ACh-induced contractions, and the inhibitory effect was not counteracted by a β-blocker. Administration of a nitric oxide synthase inhibitor or potassium channel blockers did not affect the ASRE-induced relaxation. Oral administration of ASRE for 1 and 4 d reduced the increased GI transit in CCh-treated but did not affect the GI transit of normal mice. These results indicate that ASRE exhibited dual effects of contraction via muscarinic receptors and direct relaxation on mouse ileal function, and its relaxant effect could be useful in treating diarrhea symptoms, resulting in an increase in the parasympathetic nerve activities.

Key words Acanthopanax senticosus root extract; mouse ileum; gastrointestinal motility; contraction; relaxation; diarrhea

INTRODUCTION

Gastrointestinal (GI) disorders, such as constipation and diarrhea, often occur because of factors such as lifestyle disturbances, stress, and side effects of drugs, and such unpleasant symptoms adversely affect the QOL. Irritable bowel syndrome, one of the most common functional bowel disorders, is characterized by recurrent abdominal pain, discomfort, and bloating. Physical or emotional stress or both are considered important factors that exacerbate symptoms of this syndrome. Dysfunction of the autonomic nervous system is considered one of the factors contributing to the pathogenesis of this syndrome. However, the etiology is complex and appears to be multi-factorial. Laxative agents are widely used for the treatment of GI motility disorders, including irritable bowel syndrome, but they have undesirable side effects such as nausea, abdominal pain, constipation, and diarrhea. Many supplements commonly used against GI disorders were developed from folk/herbal remedies with traditional applications, for example, peppermint oil, artichoke, and turmeric. Acanthopanax senticosus Harms, family Araliaceae (AS, previously classified as Eleutherococcus senticosus), which is commonly known as Siberian ginseng or Ciwuia, is used as a traditional Chinese medicine preparation in China, Korea, Russia, and Japan. AS is used to treat stress-induced physical and mental changes because it has anti-fatigue, neuroprotective and immunomodulatory effects. According to the Chinese Pharmacopoeia, the dried root and rhizome or stem of AS are widely prescribed to nourish the qi, fortify the spleen, tonify the kidney, and tranquilize the mind. AS root extract (ASRE) has been reported to have anxiolytic effects by regulating autonomic nervous system functions. This neural modification raises the possibility that ASRE could improve GI disorders such as constipation, which commonly occurs in irritable bowel syndrome in patients experiencing nervousness or under stress conditions. However, no reports on the effects of AS or ASRE on GI motility have been identified. Therefore, as our primary study aim, we determined whether ASRE fundamentally alters contractile smooth muscle function using an isolated mouse ileum model and an organ bath system. Furthermore, based on the in vitro experimental results, we assessed the effects of ASRE on GI motility using the GI transit method in normal mice and a carbachol (CCh)-induced diarrhea mouse model. This study may provide new strategies to treat abnormal GI mobility especially diarrhea symptoms, resulting in an increase in the parasympathetic nerve activities.

MATERIALS AND METHODS

Animals Male ICR mice (weighing 25–40 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and housed

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for at least 1 week in a temperature controlled environment (22–24°C) at 50 ± 10% humidity under a 12-h light/dark cycle before starting the experiments. They were provided standard chow (CE-2, Clea Japan Inc., Tokyo, Japan) and water ad libitum. All protocols involving animals were approved by the animal ethics committee (P-12-2018-04-A) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Mukogawa Women’s University, Japan.

Materials ASRE dry powder was provided by Sun Chlorella Co. (Kyoto, Japan, Lot No. 8142, 100% AS), which is the same product we previously used.24) The dried root tips (fresh weight: 10kg) of AS from Heilongjiang, China were extracted using HPLC and an octadecylsilyl column, according to the manufacturer’s instruction. ASRE was dissolved in distilled water for manufacturer’s instruction. ASRE was dissolved in distilled water for 100% AS, which is the same product we previously used.24) The dried root tips (fresh weight: 10kg) of AS from Heilongjiang, China were extracted using HPLC and an octadecylsilyl column, according to the manufacturer’s instruction. ASRE was dissolved in distilled water for manufacturer’s instruction. ASRE was dissolved in distilled water for manufacturer’s instruction. ASRE was dissolved in distilled water for manufacturer’s instruction.

Effects of ASRE on Isolated Mouse Ileum Contractile and relaxing ileal responses were measured using an organ bath as previously described.25) The mice were starved for 24h before the experiment but water was available ad libitum. Saline (vehicle) or CCh (1 mg·kg⁻¹) solution was subcutaneously injected. After 15 min, ASRE (900 mg·kg⁻¹) or distilled water (vehicle) was administered via oral gavage. Atropine (1 mg/kg) was subcutaneously injected in CCh-induced model mice. After 30 min, a charcoal meal (5% charcoal/10% Arabic gum) was orally administered at 10mL·kg⁻¹ body weight. Finally, after 20 min, the mice were sacrificed by cervical dislocation under anesthesia with sodium pentobarbital (65 mg·kg⁻¹, intraperitoneally (i.p.)), and the small intestines were carefully isolated and immediately rinsed with ice-cold Krebs solution. GI transit of the charcoal meal was calculated for each mouse as the percentage of the distance traveled by the charcoal meal relative to the whole length of the small intestine.

Additionally, a 4-d treatment with oral ASRE administration was performed. ASRE (900 mg·kg⁻¹·d⁻¹) or distilled water was orally administered once a day for 3. After starving the mice for 24 h, they received the treatment followed by evaluation of the GI transit on day 4.

Based on the guide for dose conversion between animals and humans using body surface area,29) the ASRE dose of 900 mg·kg⁻¹·d⁻¹ was estimated to be approximately equal to the 6 g·d⁻¹ (the conventional human dose) of ASRE in humans provided by the manufacturer.

Data Analysis All data are expressed as the means ± standard error of the mean (S.E.M). Statistical analyses of contraction or relaxation data of the isolated mouse ilea were performed using the Student’s t-test or one-way ANOVA, followed by Dunnett’s test for multiple comparisons. GI transit data were analyzed using a one-way ANOVA followed by Bonferroni’s multiple comparison test. Values were considered significant when p < 0.05. Statistical analyses were performed using GraphPad Prism® software.
RESULTS

Effects of ASRE on Isolated Mouse Ileal Motility

ASRE (10^{-5}–10^{-2} g/mL, Fig. 1A) and ACh (10^{-10}–3 \times 10^{-8} M, Fig. 1B) induced contractions in a dose-dependent manner. The maximum contractile force induced by ASRE was approximately half of that induced by ACh (Table 1). In the presence of atropine (3 \times 10^{-6} M, 3 min), a non-selective muscarinic receptor antagonist, ASRE-induced contractions at 10^{-5}–10^{-3} g/mL disappeared, while relaxation responses were observed at 3 \times 10^{-5} and 10^{-2} g/mL (Fig. 2). L-NAME did not affect the atropine-induced relaxation in the presence of ASRE (data not shown). Furthermore, neither the ATP-sensitive K\(^+\) channel blocker, Gli (10^{-3} M, 10 min), nor the BK\(_{ca}\) channel blocker, TEA (1 mM, 30 min), affected ASRE-induced relaxations (data not shown).

ACh-induced contractions of the isolated mouse ileum decreased in the presence of 3 \times 10^{-4} g/mL ASRE (Fig. 3A and Table 2). In contrast, the \(\beta\)-blocker, propranolol, had no effect on the inhibition of the ACh-induced contractions by ASRE (Fig. 3B). Furthermore, 3 \times 10^{-4} g/mL ASRE also reduced contractions induced by 40 mM KCl, while atropine (3 \times 10^{-6} M) had no effect (Fig. 3C).

Effects of Single and 4-d Oral Administration of ASRE on GI Transit of Charcoal Meal in Mice

We examined the effects of a single oral administration of 900 mg·kg\(^{-1}\) ASRE on GI transit in normal mice and a CCh-induced diarrhea mouse model (Fig. 4A). Oral administration of ASRE for 30 min did not affect the distance traveled by the charcoal meal in normal mice. On the contrary, induction of diarrhea in mice with 1 mg·kg\(^{-1}\) CCh, increased GI transit of the charcoal meal and this effect was decreased by ASRE administration. A beneficial effect was observed following treatment with 1 mg·kg\(^{-1}\) atropine, similar to that observed in previous studies.\(^5\)

Furthermore, we determined whether the effect of ASRE on normal mice or the CCh-induced diarrhea mouse model changed when the treatment was extended from 1 to 4 d (Fig. 4B). Similar to the effects of single-dose ASRE administration (Fig. 4A), multiple administrations of ASRE did not affect charcoal transport in the normal mice, but inhibited the increased GI transit in the CCh-induced diarrhea model mice (Fig. 4B).

DISCUSSION

This study demonstrated that ASRE induced dose-dependent contractions of the isolated mouse ileum and that ACh-like muscarinic receptor agonism may be involved in the contractions. In contrast, in the presence of a muscarinic antagonist, atropine, ASRE directly relaxed the mouse ileum. Furthermore, oral administration of 900 mg·kg\(^{-1}\) ASRE, which is almost the daily dose in humans, did not affect GI transit of the charcoal meal in normal mice. However, ASRE reduced the increased GI transit observed with CCh-induced diarrhea. This favorable action was observed only in the diarrhea mouse model after 1 or 4 d of treatment. These results suggested that ASRE may exhibit antidiarrheal activity by modulating GI motility.
ASRE exerted a dual action in the mouse ileum in the in vitro experiments and induced contractions to a maximum degree that was almost half of that caused by ACh. We initially measured the amount of ACh contained in ASRE using the ACh assay, as previously described, and determined that the concentration in ASRE was below the detection limit. Since the ASRE-induced contraction was completely antagonized by atropine, the ASRE probably contained an ACh-like substance, which seemed to be the main constituent that caused the ileal contraction although its concentration was very low. In contrast, unexpectedly, ASRE relaxed the mouse ilea in the presence of atropine, which antagonized muscarinic receptor(s). To further experimentally elucidated the underlying mechanism of ASRE and found that it significantly reduced ACh- and high K⁺-induced contractions, although atropine was not effective in the second scenario. These findings indicate that the ASRE may have directly relaxed the ileal smooth muscle, and did not contain an atropine-like substance.

Our previous findings demonstrated that ASRE induced endothelial nitric oxide (NO)-independent vasorelaxation of the rat thoracic aorta and raised the possibility that K⁺ channel opener(s) or Ca²⁺ channel inhibitor(s) in ASRE mediated the mechanisms underlying relaxation of the vascular smooth muscle. In fact, in the intestine, it has been revealed that adenosine plays as an inhibitory modulatory role in the contractility of the mouse ileal longitudinal muscles by opening large (BKCa) and small (SKCa) conductance Ca²⁺-activated K⁺ channels. However, the present study demonstrated that both ATP-sensitive K⁺ channel blocker (Glib) and non-specific K⁺ channel blocker (TEA) did not affect the ASRE-induced relaxations under atropine treatment in the mouse ileum. Additionally, propranolol, a non-selective β receptor antagonist, also did not affect the ASRE-induced relaxation. These findings could preclude the possible involvement of NO produced by ASRE and the presence of β agonist(s) or K⁺ channel opener(s) in the relaxation of the mouse ileum by ASRE. Another possible mechanism underlying the effect of ASRE is blockade of Ca²⁺ channels in the ileal smooth muscle, leading to inhibition of the contractile response.

Since ASRE showed dual opposing contraction and relaxation functions in the isolated mouse ileum, it was difficult to predict whether ASRE improved constipation or diarrhea. We previously demonstrated that although royal jelly contained a high concentration of ACh and induced contractions of the isolated mouse ileum in vitro, oral administration did not significantly alter GI motility in both normal mice and a loperamide-induced constipation model mouse. From these
findings, we concluded that ACh-like substances in ASRE did not increase in vivo ileal mouse motility. In contrast, we suspected that ASRE-induced relaxation could affect GI functions. Therefore, we studied the effects of oral administration of ASRE on normal mice and a CCh-induced diarrhea mouse model using the GI transit method. Oral administration of ASRE to normal mice at a dose of 900 mg·kg⁻¹·d⁻¹, which is equivalent to the daily 6 g human dose, had no effect on GI transit of the charcoal meal after a 1- or 4-d treatment. In contrast, a single administration of ASRE to the CCh-induced diarrhea mouse model decreased the enhanced intestinal motility. Furthermore, 4-d administration of ASRE improved intestinal motility, which was enhanced by CCh and its efficacy/potency did not differ from that observed after the 1-d treatment. Importantly, the ASRE-induced decrease in GI motility was specific to conditions where the intestinal motility was enhanced, as a 1- or 4-d ASRE administration did not alter the intestinal motility in mice under normal condition. In contrast, a possible transient decrease in blood pressure on the day 1 of the treatment was observed, as previously reported in rats. Furthermore, possible drug interactions should be considered when ASRE is combined with other drugs and supplements. A previous study reported that AS extract suppressed the activities of P-glycoprotein and peptide transporters in a non-competitive manner in caco-2 cells, whereas it did not affect the activity of CPY 2D6 or CYP3A4 when administered at the usual recommended dose to normal volunteers.

In the present study, we did not identify the active component of ASRE, especially the component that caused ileum relaxation. However, chlorogenic acid is one of the major components of the ASRE, according to the manufacturer’s report. Chlorogenic acid has been shown to elicit an antispasmodic effect on the rat jejunum and guinea pig ileum and dose-dependently inhibit CCh-induced contractions of the mouse bladder. In contrast, chlorogenic acid was reported to slightly increase contractile activity of the guinea pig ileum. However, there are no other reports about other components that contract or relax the ileum. Therefore, further investigations of the active components of ASRE mediating the relaxant effects on the ileum and their underlying mechanisms are necessary.

CONCLUSION

In conclusion, the ASRE investigated in this study caused in vitro contraction by interacting with muscarinic ACh receptors and direct relaxation of mouse ileal smooth muscle. In addition, 1- or 4-d oral administration of ASRE had no effect on GI transit in mice under normal conditions, whereas it decreased the enhanced intestinal motility under CCh-induced diarrhea conditions. These findings indicated that ASRE did not cause serious symptoms such as constipation under normal conditions, but it improved enhanced intestinal motility with symptoms such as diarrhea. Further investigations of the active ingredients in ASRE that contribute to ileal relaxation and the mechanisms underlying this effect are needed. However, the results of this study suggest that ASRE has potential as a new strategy for the treatment of abnormal GI motility especially diarrhea symptoms, resulting in an increase in the parasympathetic nerve activities.

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Conflict of Interest The authors declare no conflict of interest.

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