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Antihyperalgesic effects of ginseng total saponins in a rat model of incisional pain

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ABSTRACT

Background: The aim of this study was to assess whether intraperitoneal administration of ginseng total saponins (GTS) has antihyperalgesic effects in a rat model of incisional pain. The proinflammatory responses and reversal of the antihyperalgesic effect of GTS by N-methyl-D-aspartate (NMDA) or naloxone were also evaluated.

Materials and methods: Rats were injected intraperitoneally with 0.9% saline vehicle or various doses of GTS before or after a plantar incision. Paw withdrawal in response to application of the von Frey filament with the lowest bending force marked the mechanical withdrawal threshold (MWT). Blood samples were collected for the assessment of serum interleukin (IL)-1β and IL-6 levels. The IL levels were measured using an enzyme-linked immunosorbent assay kit. Rats were injected intraperitoneally with NMDA or naloxone before the GTS injection to assess the reversal of the antihyperalgesic effect of GTS.

Results: The MWT measured 2 h after the plantar incision increased significantly after the postincision administration of 50, 100, or 200 mg/kg of GTS compared with the MWT at 2 h after plantar incision. The MWT also increased significantly after the preincision injection of 100 or 200 mg/kg of GTS compared with the MWT of the vehicle control. Administration of GTS suppressed the postincision rise in serum IL-1β levels and NMDA inhibited the increase in the MWT compared with GTS alone.

Conclusions: Intraperitoneal administration of GTS before or after surgery induces antihyperalgesic effects in a rat model of incisional pain. The effects on mechanical hyperalgesia may be associated with anti-inflammatory cytokines and NMDA signaling.

1. Introduction

Ginseng, the root of *Panax ginseng* Meyer, is an herbal medicine that has been shown to exhibit a variety of medicinal effects. As part of the traditional folk medicine, ginseng has been used to alleviate toothache, abdominal pain, chest pain, and neuralgia. Ginseng saponins or ginsenosides, which have a four-ring, steroid-like structure with the attachment of sugar moieties, are the primary molecules responsible for the effects of ginseng. The properties of this chemical structure are similar to those exerted by acetylcholine, adrenaline, histamine, or opioids [1,2].

Brennan *et al.* developed a useful rat model of incisional pain that simulates human postoperative pain syndrome. In

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this model, a surgical incision through the skin, fascia, and muscle of the plantar surface of the rat hindpaw causes mechanical hyperalgesia [3]. Intrathecal ginseng total saponins (GTS) were shown to effectively alleviate the pain evoked by paw incision in a rat model [4,5], but the antihyperalgesic effects of intraperitoneal administration of GTS have not been previously established.

One of the mechanisms of postoperative pain involves the surgically induced production and release of a variety of inflammatory mediators [5,6]. Although ginseng saponins appear to induce anti-inflammatory effects [7], no previous study has investigated whether GTS can inhibit the expression of proinflammatory cytokines in a rat model of incisional pain.

This study investigated the antihyperalgesic effects of the postincision or preincision intraperitoneal injection of GTS in a rat model of incisional pain. The related effects on inflammation were quantified by measuring interleukin (IL)-1β and IL-6 production. The ability of N-methyl-D-aspartate (NMDA) or naloxone to reverse the effect of GTS on the mechanical hyperalgesic response was also investigated.

2. Materials and methods

These experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Chung-ang University (2012-0004). The animals were treated in accordance with the guidelines established by the National Institutes of Health and the International Association for the Study of Pain for the use of laboratory animals.

2.1. Animals and drugs

Adult male Sprague-Dawley rats (weight, 250–300 g) obtained from Coretec Laboratories (Seoul, Korea) were used for all experiments. The animals were allowed to habituate to the colony room for 1 wk before the experiment began. The animals were housed with two rats per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light-dark cycle. The animals were provided food and water ad libitum. The GTS was obtained from Ambo Institute (Daejon, Korea) and was a mixture of various types of individual ginseng saponins, the proportions of which are listed as follows: Rb1 (18.26%), Rb2 (9.07%), Rc (9.65%), Rd (8.24%), Re (9.28%), Rf (3.48%), Rg1 (6.42%), Rg2 (3.63%), Rg3 (4.70%), Ro (3.82%), and Ra (2.91%), and other minor ginseng saponins. All the chemicals except GTS were purchased from Sigma-Aldrich (St. Louis, MO). All drugs used for injection were dissolved in sterile saline (0.9% NaCl solution).

2.2. Surgery

For all rats, anesthesia was induced using isoflurane in pure oxygen inside an induction chamber. Once unconscious, the rats were removed and placed on a nonrebreathing anesthetic circuit with mask delivery of isoflurane in pure oxygen throughout the procedure. A subcutaneous injection of cefazolin (20 mg/kg) was given to each rat before the incision was made. Surgery was carried out as described previously [3], with minor modifications in the reported technique. Briefly, after aseptic preparation and draping, a 1-cm longitudinal skin incision was made on the plantar surface of the left hindpaw, starting 0.5 cm distal to the tibiotarsus, and extending toward the digits. The plantaris muscle was elevated and incised longitudinally, leaving the insertion and origin intact. After hemostasis, the incision was closed with two interrupted horizontal mattress sutures of 5-0 nylon. The wound was covered with antibiotic ointment (Mupirocin; Hanal Biopharma, Seoul, Korea). All rats were allowed to recover from anesthesia and surgery for a period of at least 1 h. The incisions were checked daily, and animals that exhibited any apparent wound infections or dehiscence were excluded from the study.

2.3. Drug administration and groups

To assess the potentially antihyperalgesic effects of postincision intraperitoneal injections of GTS, the rats were randomly divided into five groups ($n = 8$ in each group). At 2 h after the plantar incision, vehicle (0.9% saline) was injected into the rats in group POST C. Groups POST 25, POST 50, POST 100, and POST 200 were treated with 25, 50, 100, and 200 mg/kg of GTS, respectively, at 2 h after the plantar incision was made.

To investigate the antihyperalgesic effects of preincision intraperitoneal administration of GTS, the rats were randomly divided into five groups ($n = 8$ in each group). Vehicle (0.9% saline) was injected into the rats in group PRE C 30 min before the plantar incision was made. Groups PRE 25, PRE 50, PRE 100, and PRE 200 were pretreated with GTS injections at doses of 25, 50, 100, and 200 mg/kg 30 min before the plantar incision.

To determine whether the observed effects of GTS were antagonized by NMDA or naloxone, the rats were intraperitoneally injected with NMDA (30 μg/kg) in group NMDA ($n = 10$) or naloxone (5 mg/kg) in group NALOXONE ($n = 10$) 2 h after the plantar incision had been made and 10 min before the GTS (200 mg/kg) injection. Rats in group GTS ($n = 10$) were intraperitoneally injected with 200 mg/kg of GTS alone.

The vehicle and GTS solutions were administered at a volume of 10 mL/kg.

2.4. Pain behavioral measurements

The unrestrained rats were placed on an elevated plastic mesh floor (8 × 8-mm grid) under a clear plastic cage (21 × 27 × 15 cm) and allowed to acclimate for 15 min.

The animals were tested to determine their withdrawal thresholds to mechanical stimuli using von Frey filaments. The filaments were applied vertically to an area adjacent to the wound by using just enough pressure to bend the filament gently. Filaments with bending forces of 4, 9, 20, 59, 78, 98, 147, and 254 mN were applied in a progressively increasing manner until the hindpaw was withdrawn or a bending force of 254 mN was reached. Each filament was applied three times at an interval of 3 min. The lowest bending force that caused paw withdrawal on application of the filament determined the mechanical withdrawal threshold (MWT) of the hindpaw. After a response was observed, filaments with higher and lower bending forces were tested to confirm the MWT. The