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Research article

Antinociceptive and anti-inflammatory effects of ginsenoside Rf in a rat model of incisional pain

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ABSTRACT

Background: Ginseng saponin has long been used as a traditional Asian medicine and is known to be effective in treating various kinds of pain. Ginsenoside Rf is one of the biologically active saponins found in ginseng. We evaluated ginsenoside Rf's antinociceptive and anti-inflammatory effects, and its mechanism of action on adrenergic and serotonergic receptors, in an incisional pain model.

Methods: Mechanical hyperalgesia was induced via plantar incision in rats followed by intraperitoneal administration of increasing doses of ginsenoside Rf (vehicle, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, and 2 mg/kg). The antinociceptive effect was also compared in a Positive Control Group that received a ketorolac (30 mg/kg) injection, and the Naïve Group, which did not undergo incision. To evaluate the mechanism of action, rats were treated with prazosin (1 mg/kg), yohimbine (2 mg/kg), or ketanserin (1 mg/kg) prior to receiving ginsenoside Rf (1.5 mg/kg). The mechanical withdrawal threshold was measured using von Frey filaments at various time points before and after ginsenoside Rf administration. To evaluate the anti-inflammatory effect, serum interleukin (IL)-1β, IL-6, and tumor necrotizing factor-α levels were measured.

Results: Ginsenoside Rf increased the mechanical withdrawal threshold significantly, with a curvilinear dose–response curve peaking at 1.5 mg/kg. IL-1β, IL-6, and tumor necrotizing factor-α levels significantly decreased after ginsenoside Rf treatment. Ginsenoside Rf's antinociceptive effect was reduced by yohimbine, but potentiated by prazosin and ketanserin.

Conclusion: Intraperitoneal ginsenoside Rf has an antinociceptive effect peaking at a dose of 1.5 mg/kg. Anti-inflammatory effects were also detected.

1. Introduction

Ginseng, the root of *Panax ginseng* Meyer, is a traditional Asian herbal medicine that has been used for more than thousands of years to reduce neuralgia, toothache, abdominal pain, and chest pain [1]. Ginseng saponins, also known as ginsenosides, have a steroid-like chemical structure consisting of four rings with sugar moieties attached. Ginsenosides have biological properties similar to those of histamines, opioids, adrenaline, and acetylcholine [2]. Several experimental studies have demonstrated the antinociceptive effects of ginseng extracts in various pain models including those of abdominal, neuropathic, chronic, and incisional pain [3–7]; the mechanisms of action that have been suggested to explain this effect include antagonism of adrenergic, cholinergic, gamma-aminobutyric acid, *N*-methyl-D-aspartate, and opioid receptors [3,5,8–10].

More than 20 different ginsenosides have been found in ginseng, making them major components of this herbal medicine [11]. Among those, ginsenoside Rf, a trace ginsenoside extract (3.48%), has been shown to have an antinociceptive effect [4,5,12]. Although the antinociceptive effect of ginseng extracts has been studied previously in an incisional pain model [3,6,9], the antinociceptive effects of isolated ginsenoside Rf have not been studied in a rat incisional mode, which induces mechanical hyperalgesia through surgical incision of the plantar surface of the hind paw and thus simulates human postoperative pain [13]. In addition, ginsenoside

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Rf's antinociceptive mechanism of action is not known; it has been hypothesized to act upon adrenergic receptors, similar to other ginsenosides, or serotonin receptors, although this pathway has not been previously identified in this type of pain model.

Surgical incision and intraoperative injury induce postoperative pain by increasing central neuronal excitability leading to peripheral and central sensitization [14]. Proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrotizing factor (TNF)-α are known to exacerbate postoperative pain and mediate the pain mechanism and hyperalgesia [15–18]. If sensitization is sustained, postoperative pain may progress to chronic pain, which can lead to physical, psychological, and social disability.

The aim of the present study was to evaluate the antinociceptive effect of ginsenoside Rf and its mechanism of action, including its effects on adrenergic and serotonergic receptors in a rat incisional pain model. Proinflammatory cytokine levels, including IL-1β, IL-6, and TNF-α, were also assessed to determine the anti-inflammatory effect of ginsenoside Rf.

2. Materials and methods

Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Chung-ang University (2016-00014). The present study was performed according to the guidelines established by the National Institutes of Health, the policies of the International Association for the Study of Pain for the use of laboratory animals, and the recommended guideline in the Animal Research Reporting *In Vivo* Experiments statement [19].

Ginsenoside Rf was obtained from Ambo institute (Daejon, Korea). The HPLC purity of Rf used in the present study was 99.01%. Prazosin, yohimbine, and ketanserin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ketorolac was purchased from Hanmi Pharmaceutical Corporation (Seoul, Korea).

2.1. Animal preparation and incisional pain model

Adult male Sprague–Dawley rats weighing 250–300 g (Coretec Laboratories, Seoul, Korea) were used. They were habituated in the colony room for 1 wk before experimentation. Each cage housed with two rats at 22 ± 0.5°C with a 12:12 h light–dark cycle. Food and water were available *ad libitum*.

All the experiments were performed between 8:00 AM and 1:00 PM to avoid diurnal variation. One investigator prepared the incisional pain model as previously described [13]. Briefly, the animals were anesthetized with isoflurane (Aerane Solution; Ilsung-medicine, Seoul, Korea) using a chamber for induction and a nonrebreathing circuit system with mask delivery for maintenance. After subcutaneous injection of cefazolin (Cefazoline, 20 mg/kg; Chong Kun Dang Pharmaceutical Corporation, Seoul, Korea) and aseptic draping, a 1-cm skin incision was made in the area between 0.5 cm distal to the tibiotarsus and digits on the plantar area of the left hind paw. The plantaris muscle was then elevated, and an incision was made longitudinally, keeping the muscle origin and insertion site intact. The skin was sutured with 5-0 nylon, and prophylactic antibiotic salve (Mupirocin; Hanal Biopharma, Seoul, Korea) was applied (Fig. 1). Lesions were checked daily, and rats with suspected wound dehiscence or infection were excluded.

2.2. Drug administration

Dose response test Fifty rats were randomly divided into five groups to evaluate the antinociceptive effect of different doses of ginsenoside Rf. Two h after the incision, each rat in the four ginsenoside Rf dosing groups (Rf 0.5, Rf 1, Rf 1.5, and Rf 2) as well as Group C (the control) were injected with ginsenoside Rf (0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, or 2.0 mg/kg) or 0.9% saline vehicle, respectively (Fig. 2). Ginsenoside Rf was dissolved in distilled water with an intraperitoneal (IP) injection volume of 10 mL/kg. Each solution was prepared in opaque syringes with sequential number according to a randomization list generated by an investigator who was not involved in any other stages of the study. Random sequence generation was performed using PASS 11 software (NCSS, Kaysville, UT, USA).

Positive control and naïve group To assess the validity of the present study, the antinociceptive effect in the dosage (1.5 mg/kg) of ginsenoside Rf was compared with that in a positive control group receiving an analgesic and in the Naïve Group. The Positive Control Group (Group Keto 30, $n = 10$) was administered the nonsteroidal anti-inflammatory analgesic ketorolac (30 mg/kg IP) [20]. The Naïve group ($n = 10$) were not incised and were administered 0.9 % saline IP.

Mechanism test for the antinociceptive effect To examine whether the observed effects of ginsenoside Rf on mechanical hyperalgesia induced by plantar incision were mediated by either adrenergic (α_1, α_2) or serotonergic (5-HT_{2A}) receptors, studies with specific receptor antagonists were utilized. Prazosin (1 mg/kg, α_1 adrenergic receptor antagonist, $n = 10$), yohimbine (2 mg/kg, α_2 adrenergic receptor antagonist, $n = 10$), ketanserin (1 mg/kg 5-HT_{2A} receptor antagonist, $n = 10$), or saline was administered by IP injection 110 min after a skin incision was made in the hind paw. After 10 min, ginsenoside Rf (1.5 mg/kg) was injected into the peritoneal cavity at a volume of 10 mL/kg (Fig. 2). The drug dosage levels were based on previous studies on antinociceptive mechanisms of action [21–24].

2.3. Pain behavioral test

The nociceptive threshold was measured using von Frey filaments. The rats were placed individually in a clear Plexiglas cage (21 × 27 × 15 cm) with a plastic grid floor (8 × 8 mm). Mechanical hyperalgesia was assessed via bending force in ascending order (4mN, 9mN, 20mN, 59mN, 78mN, 98mN, 147mN, and 254mN). The von Frey filaments were applied vertically to the midplantar surface of the incisional hind paw for 5 s or until there was a positive response. A positive response was noted when the rats showed a rapid withdrawal or flexion of the stimulated hind paw. The mechanical withdrawal threshold (MWT) was determined via the lowest bending force, and confirmed by additionally applying higher and lower bending forces than the MWT. If a positive response was not observed at 254mN, the bending force was considered the MWT. The MWT measurement was conducted at the following time points after ginsenoside Rf administration: 1 d before the incision (BI); 2 h after plantar incision (AP); 15 min, 30 min, 45 min, 60 min, 80 min, and 120 min; 24 h and 48 h after ginsenoside Rf administration (Fig. 2). All behavioral assessments and animal experiments were carried out by an expert investigator blinded to the groups.

2.4. Measurement of IL-1β, IL-6, and TNF-α levels

Blood samples from the tails of the rats were collected 1 d BI, 120 min, and 24 h and 48 h after administration of ginsenoside Rf or vehicle control (Fig. 2). Blood samples were centrifuged at 1,000 *g* for 20 min, and the supernatants were stored at −80°C until analysis. IL-1β, IL-6, and TNF-α levels were measured using a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (R&D systems, Minneapolis, MN, USA). Briefly, the 96-well plate was coated with each anticytokine capture antibody (Ab). If the related cytokine was

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