General pharmacological profiles of bee venom and its water soluble fractions in rodent models

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Recently, the antinociceptive and anti-inflammatory efficacy of bee venom (BV, Apis mellifera) has been confirmed in rodent models of inflammation and arthritis. Interestingly, the antinociceptive and anti-inflammatory effect of whole BV can be reproduced by two water-soluble fractions of BV (>20 kDa: BVAF1 and <10 kDa: BVAF3). Based on these scientific findings, BV and its effective water-soluble fractions have been proposed as potential anti-inflammatory and antinociceptive pharmaceuticals. While BV’s anti-inflammatory and antinociceptive properties have been well documented, there have been no careful studies of potential, side effects of BV and its fractions when administered in the therapeutic range (BV, 5 µg/kg; BVAF1, 0.2 µg/kg; BVAF3, 3 µg/kg; subcutaneous or intradermal). Such information is critical for future clinical use of BV in humans. Because of this paucity of information, the present study was designed to determine the general pharmacological/physiological effects of BV and its fractions administration on the rodent central nervous, cardiovascular, respiratory and gastrointestinal system. Subcutaneous BV and its fractions treatment did not produce any significant effects on general physiological functions at the highest dose tested (200-fold and 100-fold doses higher than that used clinically, respectively) except writhing test. These results demonstrate that doses of BV or BV subfractions in the therapeutic range or higher can be used as safe antinociceptive and anti-inflammatory agents.

Key words: bee venom, general pharmacology, antinociception, anti-inflammatory

Introduction

For several centuries, bee venom (BV) of Apis mellifera has been used in oriental medicine to treat a number of inflammatory diseases including tendonitis, bursitis and rheumatoid arthritis [1]. BV therapy has been considered as an alternative to more traditional acupuncture and moxibustion therapy. Recently, we have demonstrated that BV therapy also produces potent therapeutic effects on osteoarthritis [7]. Subsequently, the anti-inflammatory and antinociceptive effects of BV were further verified using several animal models with acute and chronic nociception. For example, subcutaneous treatment of BV produced a dramatic anti-inflammatory and antinociceptive effect on Freund’s adjuvant-induced arthritis in rats [8]. In addition, subcutaneous BV treatment significantly suppressed the paw edema and hyperalgesia associated with carrageenan-induced acute inflammation in rats [11]. Moreover, subcutaneous BV treatment produced significant visceral antinociception in mice following abdominal acetic acid injection [6] and it suppressed pain behaviors and spinal Fos expression in rats induced by hindpaw formalin injection [5]. As a crucial step towards determining the specific antinociceptive and anti-inflammatory components of BV, whole BV constituents were fractionized according to their solubility (i.e. water-, ethylacetate-, and hexane-soluble fractions) and subsequently tested for their antinociceptive and anti-inflammatory properties. The results of this study indicated that the water-soluble fraction of BV (BVA) is responsible for producing BV’s anti-inflammatory and antinociceptive effects in a rodent model of rheumatoid arthritis [9]. BVA contains high molecular weight enzymes (glycoproteins >20 kDa) including phospholipase A₂ and hyaluronidase as well as low molecular weight polypeptides.
(≤10 kDa) that include melittin, apamin, adolapin and mast cell degranulating (MCD) peptide [10]. There appear to be fewer constituents with molecular weights between 10 and 20 kDa in whole BV and these substances have not been well characterized. BVA has been purified in our laboratory and separated into the following three molecular weight fractions: BVAF1 (>20 kDa), BVAF2 (<20 kDa and ≥10 kDa), and BVAF3 (<10 kDa). Each fraction has been tested for pharmacological efficacy in previous studies in our lab. The results of this study indicate that subcutaneous injection of the BVAF1 and BVAF3 fractions produce the greatest suppressive effect on Freund’s adjuvant-induced paw edema and on the mechanical/thermal hyperalgesia associated with Freund’s adjuvant-induced inflammation in rats. In addition these two fractions alleviated radiological changes (i.e. bone proliferation and soft tissue swelling) in rat model with joint arthritis.

Despite the accumulating evidence showing a profound antinociceptive and anti-inflammatory effect of subcutaneous BV and BVA treatment, there have been very few studies that have examined the effect of BV or BVA therapy on a variety of physiological systems. Such information is important with respect to drug safety issues and is critical for the predicted increasing use of BV or its fractions for treating human patients. Because of the paucity of information related to these issues, the present study was designed to investigate the general pharmacological effects of BV, BVAF1 and BVAF3 on several physiological parameters of the central nervous, digestive, cardiovascular and respiratory systems in rodents.

Materials and Methods

Test reagents

Bee venom (BV) of *Apis mellifera* was purchased from Sigma (USA). The water-soluble fraction of BV was partitioned from whole BV and the water-soluble partition was subsequently fractionated by molecular weight into BVAF1 (>20 kDa) and BVAF3 (<10 kDa) using Minitan Filter plates (Millipore, USA) as previously described [12]. Each fraction was completely dried and then stored at refrigerator temperature. A single clinical dose of BV is 5 µg/kg when administered by either an intradermal or subcutaneous route in human patients in Korea. Since BV subfractions have not been administered to human patients, the theoretical dose was calculated by considering the partial ratio of the subfractions to whole BV. Based on this ratio we calculated the clinical dose of the BVAF1 and BVAF3 subfractions to be 0.2 µg/kg and 3 µg/kg, respectively. BV and the BVAF1 and BVAF3 subfractions were dissolved in saline and then administered subcutaneously to the animals in each experimental group. In order to examine dose-response characteristics, a high dose of BV or its fractions was selected in terms of the range from 10-fold to 100-fold the effective clinical dose.

Acetic acid and atropine sulfate were purchased from Fluka (CH-9471, Buchs, Switzerland). Acetylsalicylic acid, aminopyrine, activated charcoal, chlorpromazine HCl and sodium pentobarbital were purchased from Sigma (USA). These positive drugs were administered simultaneously with vehicle or with BV or its subfractions. A standard physiological saline solution was used as the vehicle for all experiments.

Animals

These experiments were performed on male ICR mice (25-30 g), Sprague-Dawley rats (200-300 g) and New Zealand White rabbits (2-2.5 kg). All laboratory animals were obtained from the Hallym Laboratory of Animal Sciences (Korea). The protocol for animal care used in the present study were approved by the Animal Care and Use Committee at Seoul National University and its methodology conforms to the published guidelines of the USA National Institutes of Health (NIH publication No. 86-23, revised 1985). In addition, the ethical guidelines of the International Association for the Study of Pain for investigating experimental pain in conscious animals were also followed [17]. Animals were housed under the conditions of constant temperature (23 ± 2°C), relative humidity (55 ± 5%), and light/dark cycle (12 h/12 h: illumination at 7:00 AM) until the day of the experiment (a minimum 7 day acclimation period).

Effect of BV fractions on the central nervous system

General behavior in mice: Each dose of BV, BVAF1 or BVAF3 was administrated subcutaneously in separate groups of ICR mice (total n = 90). In the control group, physiological saline was injected into corresponding site. Two experimenters, blinded to the animal treatment, observed and recorded details of behavior at 5, 15, 30, 60, 120, 180 min and 24 h after BV or saline treatment using a modification of the approach described by Irwin [3]. Animals were checked daily for mortality, gross signs of toxicity and abnormal behavior for 7 days post-treatment.

Sleep-induction time and duration in mice: Vehicle, and BV, BVAF1 and BVAF3 were administered subcutaneously 30 min prior to sleep induction (total n = 90 mice; n = 10 mice/group). Sodium pentobarbital (32 mg/kg), sedative/anesthetic drug was injected intraperitoneally in each group of mice to induce sleep. One group of mice (n = 10) was intramuscularly injected with chlorpromazine HCl (1 mg/kg) as a positive control because the aliphatic phenothiazine drugs, such as chlorpromazine, are highly sedative. The effect of different doses of BV, BVAF1 and BVAF3 on sleep induction time and on sleep duration produced by sodium pentobarbital was subsequently analyzed. The loss of the