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Original article

The inclusion complex of carvacrol and β-cyclodextrin reduces acute skeletal muscle inflammation and nociception in rats

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ABSTRACT

Background: Skeletal muscle inflammation is strongly associated with pain and may impair regeneration and functional recovery after injury. Since anti-inflammatory and antinociceptive effects have been described for the inclusion complex of carvacrol and β-cyclodextrin (βCD-carvacrol), this study investigated the effects of βCD-carvacrol in a model of acute skeletal muscle inflammation.
Methods: Muscle injury was induced in male Wistar rats by injection of 3% carrageenan in the gastrocnemius muscle. Rats were orally pretreated with saline (vehicle) or βCD-carvacrol (20, 40, 80 and 180 mg/kg) one hour before administration of carrageenan.
Results: The injection of carrageenan in the gastrocnemius muscle increased tissue myeloperoxidase (MPO) activity ($p < 0.001$), edema ($p < 0.001$) and the levels of tumoral necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, macrophage inflammatory protein (MIP-2), but not IL-10 levels. Also, it increased mechanical hyperalgesia and decreased the grip force of animals. Pretreatment with βCD-carvacrol (80 or 160 mg/kg) significantly decreased muscle MPO activity and edema 24 h after injury in comparison to vehicle-pretreated group. Animals pretreated with βCD-carvacrol (160 mg/kg) presented significantly lower levels of IL-1β, IL-6 and MIP-2 and higher levels of IL-10 six hours after induction and lower levels of TNF-α and MIP-2 after 24 h when compared to the vehicle group. Pretreatment with βCD-carvacrol also reduced mechanical hyperalgesia and limited the decrease of grip force (80 or 160 mg/kg; $p < 0.001$) 6 and 24 h after injury.
Conclusion: These results show that βCD-carvacrol reduces inflammation and nociception in a model of acute injury to skeletal muscles.

Introduction

Skeletal muscle injury can be caused by a variety of stimuli, such as trauma, chemicals, myotoxins, ischemia and diseases, which can lead to substantial tissue damage producing pain, sensory disorders, muscle dysfunction, incapacity of self-repair and the chronic motor disability [1,2].

It has been shown that skeletal muscle injury is strongly regulated by coordinated stages of degeneration, inflammation, regeneration and fibrosis. In particular, inflammation is strongly associated with pain and can impair the regeneration and functional recovery of injured skeletal muscles [1,3]. Among the experimental models of skeletal muscle injury, the gastrocnemius injury induced by injection of carrageenan is a simplified model of musculoskeletal inflammation and nociception, characterized by delayed-onset muscle soreness, reduction in grip force and development of primary and secondary hyperalgesia. It is used as model of acute muscular inflammatory pain and chronic inflammation [4].

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In recent years, many studies have been carried out to discover effective therapeutic agents and to improve clinical outcomes assessed by conservative treatment of skeletal muscle injury composed of RICE (rest, ice, compression and elevation), nonsteroidal anti-inflammatory drugs (NSAIDs) and physical therapy [5]. However, in many clinical situations, these therapies are insufficient to treat muscular inflammation and ensure adequate tissue recovery [2]. In this context, carvacrol (5-isopropyl-2-methylpheno), a phenolic monoterpene constituent of essential oils produced by numerous aromatic plants and spices such as *Nigella sativa L., Origanum majorana L, Origanum vulgare L.* and *Thymus vulgaris L.* appears to be a promising therapeutic agent for treatment of muscular injury [6]. Previous studies have reported a large range of biological effects of carvacrol, such as anti-inflammatory, analgesic and antithrombotic effects which are attributed, at least in part, to its inhibitory effects on one or both of the cyclooxygenases (COXs), as well as the inhibition of nuclear factor (NF)-κB and mitogen-activated protein kinases (MAPK) signaling pathways or the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) [7–9]. Furthermore, other important carvacrol-related biological activities, including antimicrobial, antioxidant and inhibition of acetylcholinesterase activity have been reported [10].

Nonetheless, essential oil's components have limited water solubility, which leads to the use of high doses in various experimental interventions, in turn compromising their safety and therapeutic efficacy [14]. In order to overcome this problem, β-cyclodextrins (βCD) have been used to produce inclusion complexes with lipophilic drugs and increase their aqueous solubility [11]. This has also been shown for carvacrol in experimental protocols involving orofacial and cancer-associated pain [12,13].

Based on both the need for new alternatives to treat inflammatory muscle injury and the potential of carvacrol for this activity, the hypothesis investigated in this study was that the inclusion complex of β-cyclodextrin and carvacrol (βCD-carvacrol) can impair the inflammatory and nociceptive alterations in the experimental model of carrageenan–induced acute skeletal muscle inflammation.

Materials and methods

Animals

All experimental and euthanasia procedures were performed according to the National Institutes of Health guide for the care and use of Laboratory animals, as well Brazil's National Council for the Control of Animal Experimentation (CONCEA). Protocols were approved by the Research Ethics Committee of our Institution under nº.02/2012.

Experiments were performed with male Wistar rats weighing 220–270 g, obtained from the Animal Center of our Institution. Free access to standard chow and tap water was allowed and animals were kept at room temperature (22 ± 2 °C) with a light/dark cycle of 12/12 h.

Preparation of βCD-carvacrol

The βCD-carvacrol was prepared by the slurry complex method according to Menezes et al. [14]. First, β-cyclodextrin (βCD; 1135 mg) was dissolved in distilled water (20 mL) at 70 °C. After the βCD solubilization, carvacrol (150 mg) was slowly added to the βCD solution with continuous stirring for 36 h at room temperature. Afterwards the solution was kept at desiccator until all the water was removed. Then, the sample was crushed and kept in the desiccator until analysis. Carvacrol and βCD were purchased from Sigma (St. Louis, MO, USA).

Induction of muscle inflammation

Animals were anesthetized with 3% isoflurane and given a single injection of 100 μL of 3% λ-carrageenan (Sigma, St. Louis, MO, USA) dissolved in sterile saline, in the left gastrocnemious muscle as previously described by Radhakrishnan et al. [15]. Six or 24 h after intramuscular injection, all animals were euthanized by an overdose isoflurane (5%) and gastrocnemius muscle samples were collected for subsequent measurements.

Experimental design

Before starting the experiment, the animals were randomly divided into the following groups, with 8 rats in each group:

Saline group: Rats were pretreated with saline (0.9% NaCl, 10 mL/kg, gavage) 1 h prior to intramuscular administration of 100 μL of saline in the left gastrocnemius muscle.

Carrageenan group: Rats were pretreated with saline (10 mL/kg, gavage) 1 h prior to intramuscular administration of 100 μL of 3% carrageenan in the left gastrocnemius muscle.

Dexamethasone group: Rats were pretreated with dexamethasone (2 mg/kg, 1 mL/kg, subcutaneous injection) 0.5 h prior to intramuscular administration of 100 μL of 3% carrageenan in the left gastrocnemius muscle.

βCD-carvacrol groups: Rats were pretreated with βCD-carvacrol (20, 40, 80 or 160 mg/kg, dissolved in saline, 10 mL/kg, gavage) 1 h prior to intramuscular administration of 100 μL of 3% carrageenan in the left gastrocnemius muscle.

Preliminary experiments performed in our laboratory showed that the pre-treatment of animals with βCD alone (160 mg/kg) did not interfere with carrageenan-induced nociceptive parameters or inflammatory markers.

Measurement of nociceptive parameters

The mechanical hyperalgesia was evaluated with electronic von Frey apparatus (Insight Ltda, Ribeirão Preto, Brazil) immediately before the treatments and 6 or 24 h after carrageenan injection in the gastrocnemius muscle belly. This apparatus allowed the application of an increasing force on the hind paw until withdrawal behavior was observed. Before the experiment, animals were acclimatized during 3 days, by placement for 30 min each day in plastic cages (30 × 25 x 20 cm). On the day of the experiment, before any manipulation, animals were placed in the cages for 20 min and the threshold force (from 0.1 to 1000 g) needed to evoke withdrawal behavior was quantified as the mean of three subsequent stimulations with a minimum interval of 1 min between them. This procedure was repeated 6 and 24 h after carrageenan injection. The investigator was unaware of the group's identities. Data were expressed as the variation (Δ) in the threshold stimulus (g), calculated by subtracting the basal value from the time point value for each animal.

The motor performance was evaluated by using a grip strength meter (Insight, Ribeirão Preto, SP, Brazil). Grip strength was recorded when the animal held on a plate was carefully pulled by the tail. The value registered was the highest value obtained from three consecutive trials with a minimum interval of 5 min between them [16]. The investigator was also unaware of group identity.

Inflammatory markers

Myeloperoxidase activity was measured in gastrocnemius muscle samples 24 h after carrageenan injection, according to the method described by Bradley et al. [17] with minor modifications. Briefly, muscle samples were homogenized (1:10 w/v) in 50 mmol/L phosphate buffer (pH 6.0) containing