Biomedicine & Pharmacotherapy 84 (2016) 454–461

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

Original article

Enhancement of orofacial antinociceptive effect of carvacrol, a monoterpene present in oregano and thyme oils, by β-cyclodextrin inclusion complex in mice

Juliane C. Silva[a,b], Jackson R.G.S. Almeida[b], Jullyana S.S. Quintans[b], Rajiv Gandhi Gopalsamy[b,c], Saravanan Shanmugam[b,c], Mairim Russo Serafini[c], Maria R.C. Oliveira[d], Bruno A.F. Silva[d], Anita O.B.P.B. Martins[d], Fyama F. Castro[d], Irwin R.A. Menezes[d], Henrique D.M. Coutinho[d], Rita C.M. Oliveira[e], Parimelazhagan Thangaraj[f], Adriano A.S. Araújo[c,*], Lucindo J. Quintans-Júnior[b,*]

[a] Center for Studies and Research of Medicinal Plants, Federal University of San Francisco Valley, 56.304-205 Petrolina, Pernambuco, Brazil
[b] Department of Physiology (DFS), Federal University of Sergipe, 49.100-000 São Cristóvão, Sergipe, Brazil
[c] Department of Pharmacy (DFA), Federal University of Sergipe, 49.100-000 São Cristóvão, Sergipe, Brazil
[d] Department of Biological Chemistry, Regional University of Cariri, Crato, Ceará, Brazil
[e] Department of Biophysics and Physiology, Federal University of Piaui, 64.049-550 Teresina, PI, Brazil
[f] Bioprospecting Laboratory, Department of Botany, Bharathiar University, Coimbatore, 641 046, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 16 September 2016
Accepted 17 September 2016

Keywords:
Carvacrol
Monoterpene
Cyclodextrin
Orofacial pain
Opioid
Pain

ABSTRACT

Orofacial pain is associated with diagnosis of chronic pain of head, face, mouth, neck and all the intraoral structures. Carvacrol, a naturally occurring isoprenoid with diverse class of biological activities including anti-inflammatory, analgesic, antitumor and antioxidant properties. Now, the antinociceptive effect was studied in mice pretreatment with carvacrol (CARV) and β-cyclodextrin complex containing carvacrol (CARV-βCD) in formalin-, capsaicin-, and glutamate- induced orofacial nociception. Mice were pretreated with vehicle (0.9% Nacl, *p.o.*), CARV (10 and 20 mg/kg, *p.o.*), CARV-βCD (10 and 20 mg/kg, *p.o.*) or MOR (10 mg/kg, *i.p.*) before the nociceptive behavior induced by subcutaneous injections (s.c.) of formalin (20 μl, 2%), capsaicin (20 μl, 2.5 μg) or glutamate (20 μl, 25 μM) into the upper lip respectively. The interference on motor coordination was determined using rotarod and grip strength meter apparatus. CARV-βCD reduced the nociceptive during the two phases of the formalin test, whereas CARV did not produced the reduction in face-rubbing behavior in the initial phase. CARV-βCD (20 mg/kg, *p.o.*) produced 49.3% behavior pain while CARV alone at 20 mg/kg, *p.o,* produced 28.7% of analgesic inhibition in the second phase of formalin test. CARV, CARV-βCD and Morphine (MOR) showed a significant reduction against nociception caused by capsaicin or glutamate injection. Thus the encapsulation of carvacrol in β-cyclodextrin can acts as a considerable therapeutic agent with pharmacological interest for the orofacial pain management.

1. Introduction

Orofacial pain disorders represent a tournament since the orofacial region is complex and recognized to be one of the most densely innervated areas of the body [1]. Orofacial pain can arise from many sources and are known to have depressing impact on the person feeling it; that considered to be one of the most important issues in the area of medicine [2]. According to American Academy of Orofacial Pain (AAOP), the chronic painful condition arises from different structures including teeth, sinuses,

Abbreviations: AAOP, According to American Academy of Orofacial Pain; CDs, cyclodextrins; CARV, carvacrol; CARV-βCD, β-cyclodextrin complex containing carvacrol; βCD, β-cyclodextrin; MOR, morphine; NAL, naloxone; *i.p*, intraperitoneal injection; *p.o*, oral administration; RR, reuthenium red; *s.c*, subcutaneous injection; DZP, Diazepam; TRP, Transient receptor potential.

* Corresponding authors.
E-mail addresses: adriasa2001@yahoo.com.br (A.A.S. Araújo), lucindo@pq.cnpq.br, lucindojr@gmail.com (L.J. Quintans-Júnior).

http://dx.doi.org/10.1016/j.biopha.2016.09.065
0753-3322/

eyes, nerves, blood vessels, temporomandibular joint and masticatory muscles [3].

Orofacial pain can be difficult to control with available modern drugs, therefore an alternate and safe approaches were designed to develop a bioactive molecules from natural resources that can be used as a strategy for discovering novel phytomedicines to combat chronic facial pain [4,5]. Essential oils are also known as volatile oils and are generally extracted from plant sources that played curative effects against broad spectrum of diseases, its composition depends upon seasonal variations and genetic structures of the plant [6,7]. Carvacrol (5-isopropyl-2-methylphenol) is an isoprenoid present in extensive variety of medicinal and aromatic plants, mainly in the essential oils of genera *Origanum* and *Thymus* belongs to Lamiaceae family [8,9]. Carvacrol, a phenolic monoterpene has broad pharmacological activities such as anti-inflammatory, analgesic, antitumor, antinociceptive to orofacial pain and antioxidant properties evidenced by earlier scientific reports [10–17].

However, the use of carvacrol from essential oils is very limited due to its volatility and low water solubility [18]. Cyclodextrins (CDs) are family of compounds made up of sugar molecules bound together in a cyclic ring which promotes improvements in solubility and the bioavailability of polar compounds, as terpenes [19–21]. It forms inclusion complexes mainly with volatile compounds derived from essential oils protecting its external oxidation, chemical stability and claims for its efficacy for therapeutic applications [21,22]. CDs are non-toxic soluble bioavailable dietary compound; it has a broad impacts on food, agriculture, pharmaceutical and chemical industries [20,23]. Based on these observations, we aimed to study the antinociceptive effect of the carvacrol (CARV) and β-cyclodextrin containing carvacrol (CARV-βCD) complex in formalin-, capsaicin-, and glutamate-induced orofacial nociception in mice. Furthermore, our study was designed to investigate the βCD complexed with CARV could improve the pain responses compared to CARV alone in the orofacial pain animal models.

2. Materials and methods

2.1. Preparation of inclusion complex

Preparation and physical-chemical characterization of the CARV-βCD complex were done as described previously [17].

2.2. Animals

Adult male Swiss mice of 3 months old (25–35 g) were used in this study. The animals were randomly housed in appropriate cages and divided into respective groups of six animals each. They were maintained at controlled temperature (22 ± 2 °C) under 12 h light/dark cycles with free access to food and water. Experimental protocols were approved by the regulatory bodies of the Institutional Animal Care and Use Committee (CEPA/UFS # 43/09) at the Federal University of Sergipe. All the experiments consisting the behavioral analysis were carried out blindly.

2.3. Formalin test

Orofacial nociception was induced in mice using the method described by Luccarini et al. [24] with slight modifications according to our laboratory protocol [25]. Formalin 20 μL (2.0% in 0.9% saline) was injected subcutaneously at the right upper lip (perinasal area), to assess the effects of test drugs in mice groups (n = 6, per group). The animals were pretreated systemically with vehicle (saline 0.9%, *p.o.*), CARV (10 and 20 mg/kg, *p.o.*), CARV-βCD (10 and 20 mg/kg, *p.o.*) or MOR (10 mg/kg, *i.p.*) respectively 1 h before the local injection of formalin. The algic response was measured for 40 min that was basically divided into two phases, the first phase (neurogenic) measured for 5 min immediately after formalin injection and the second phase (inflammatory) evaluated between 15 and 40 min after formalin injection. The pain was quantified at these time periods by measuring the time(s) that the animals spent face-rubbing in the injected area with its fore-or hind paws. The percent inhibition by an antinociceptive agent was determined using the equation as described earlier [26].

2.4. Capsaicin test

Orofacial nociception was induced by capsaicin as described earlier for formalin test. Capsaicin was dissolved in ethanol, dimethyl sulfoxide and distilled water (1:1:8) [27] and it was injected 20 μL (2.5 μg, *s.c.*) in the perinasal area. The experiments were carried out as follows (n = 6 per group). Group I was treated with saline (0.9%, *p.o.*), Group II and III were treated with CARV (10 and 20 mg/kg, *p.o.*), group IV and V were treated with CARV-βCD (10 and 20 mg/kg, *p.o.*) and group VI was treated with MOR (10 mg/kg, *i.p.*). The drugs were administered to animals 1 h before capsaicin injection. The quantification of nociception was performed by measuring the time intervals (s) that the animal spent on face-rubbing in the injected area with its fore or hind paws [28].

2.5. Glutamate test

Orofacial nociception induced by glutamate to mice was similar to that of formalin and capsaicin. The glutamate (20 μL, 25 μM, *s.c.*) was injected in the perinasal area in an attempt to provide evidence for the CARV and CARV-βCD interaction with the glutamatergic system. The effects of CARV (10 and 20 mg/kg, *p.o.*), CARV-βCD (10 and 20 mg/kg, *p.o.*) or MOR (10 mg/kg, *i.p.*) were evaluated according to the same experimental design as postulated previously in capsaicin test. The animals were observed individually during 15 min after the algic agent and their nociception was quantified by measuring the time (s) spent rubbing their faces in the injected area with their fore or hind paws [15].

2.6. Participation of transient receptor potential vanilloid system and opioid system in nociception

In an attempt to provide the interaction of the CARV-βCD with TRPV, it was investigated to antagonize the Capsaicin-induced orofacial nociception in mice with Ruthenium Red [29]. The procedure was similar to that previously described [25]. A volume of 20 μL (2,5 μg, *s.c.*) capsaicin was injected into the right upper lip, mice were observed individually for 20 min. Quantification of nociception was performed by measuring the time (s) that the animal spent face-rubbing in the injected area with its fore or hind paws.

The participation of the opioid system in the antinociceptive effect of CARV-βCD was examined by injecting naloxone hydrochloride (2 mg/kg *s.c.*), a non-selective opioid receptor antagonist. The animals were pretreated with NAL 15 min before the administration of CARV-βCD (20 mg/kg, *p.o.*). The other groups of animals received only CARV-βCD (20 mg/kg, *p.o.*), MOR, NAL (Naloxone), or saline 30 min before the formalin injection [25]. Nociception was quantified at these periods by measuring the time (s) when the animals spent face-rubbing in the injected area with its fore- or hind paws.

2.7. Rota rod test

A rotarod (Insight, Brazil) apparatus was used to investigate if the treatments could influence the motor activity of the animals.