Phytomedicine 21 (2014) 356–362

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

The oral administration of trans-caryophyllene attenuates acute and chronic pain in mice

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ARTICLE INFO

Article history:
Received 16 July 2013
Accepted 9 August 2013

Keywords:
Medicinal plants
Trans-caryophyllene
Opioid system
Endocannabinoid system
Interleukin 1β
Pain

ABSTRACT

Trans-caryophyllene is a sesquiterpene present in many medicinal plants' essential oils, such as *Ocimum gratissimum* and *Cannabis sativa*. In this study, we evaluated the antinociceptive activity of trans-caryophyllene in murine models of acute and chronic pain and the involvement of trans-caryophyllene in the opioid and endocannabinoid systems. Acute pain was determined using the hot plate test (thermal nociception) and the formalin test (inflammatory pain). The chronic constriction injury (CCI) of the sciatic nerve induced hypernociception was measured by the hot plate and von Frey tests. To elucidate the mechanism of action, mice were pre-treated with naloxone or AM630 30 min before the trans-caryophyllene treatment. Afterwards, thermal nociception was evaluated. The levels of IL-1β were measured in CCI-mice by ELISA. Trans-caryophyllene administration significantly minimized the pain in both the acute and chronic pain models. The antinociceptive effect observed during the hot plate test was reversed by naloxone and AM630, indicating the participation of both the opioid and endocannabinoid system. Trans-caryophyllene treatment also decreased the IL-1β levels. These results demonstrate that trans-caryophyllene reduced both acute and chronic pain in mice, which may be mediated through the opioid and endocannabinoid systems.

Introduction

Trans-caryophyllene is a bicyclical sesquiterpene and is one of the major active principles from *Cannabis sativa* (Malingré et al., 1975), *Ocimum gratissimum* (Silva et al., 1999) and *Cordia verbenaceae* (Carvalho Junior et al., 2004). It is widely used in seasoning mixtures, in various food products and in soaps and detergents (Sabulal et al., 2006).

Studies have demonstrated that trans-caryophyllene possess biological activities. For example, evidence from several experimental models show that trans-caryophyllene itself or trans-caryophyllene-containing plants possess antioxidant (Alma et al., 2003), antibacterial (Michielin et al., 2009), gastroprotective (Tambe et al., 1996), anxiolytic (Galdino et al., 2012), anti-inflammatory (Medeiros et al., 2007) and anesthetic (Ghelardini et al., 2001) activities. Trans-caryophyllene also exhibits neuroprotective effects (Chang et al., 2007) and augments the number of *natural killer* cells (Standen et al., 2006).

In addition to its biological functions, trans-caryophyllene has been shown to act as an agonist at the type 2 endocannabinoid receptors (Gertsch et al., 2008). Because the endocannabinoids play an important role in pain modulation, trans-caryophyllene may hold promise as a new pain treatment. Therefore, we evaluated the antinociceptive potential of this naturally occurring compound in acute and chronic pain models. In addition, we examined the possible mechanism of action of trans-caryophyllene by using opioid and cannabinoid antagonists and by measuring the IL-1β levels.

Materials and methods

Animals

Male C57BL/6J mice (3 months old and weighing 25–30 g) from the Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME – UNIFESP) vivarium were used. Animals were housed in rooms with controlled temperature (22 ± 1 °C) and a 12 h light/dark cycle (lights on at 7:00 a.m.). Water and food were available *ad libitum*. A minimal number of experimental animals were utilized, and animals were used only once. The C57BL/6J strain was selected because of their higher sensitivity to behavioral tests and lower inter-animal variability compared with other inbred strains (Mogil et al., 2006; Balter and Dykstra, 2013). All

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http://dx.doi.org/10.1016/j.phymed.2013.08.006

studies were conducted ethically in accordance with the Research Ethical Committee of UNIFESP (protocol #0675/09). The number of animals ($n = 5$) and the intensity of painful stimuli were as small as needed to consistently demonstrate the analgesic effect of the drug (Zimmermann, 1983).

Drugs

Trans-caryophyllene was purchased from Sigma (St. Louis, USA) and diluted in corn oil. Formaldehyde and morphine were obtained from Merck (Darmstadt, Germany) and diluted in saline. The opioid antagonist naloxone (Sigma) was also diluted in saline. The cannabinoid antagonist AM630 and agonist JWH-015 (Sigma) were diluted in a solution containing saline and dimethyl sulfoxide (90/10%). Drugs were freshly prepared on the day of each experiment and administered intraperitoneally (i.p.) or orally (p.o.) in a volume of 0.1 ml/10 g body weight.

Motor coordination (rotarod)

The motor coordination of the mice was evaluated on the rotarod apparatus at a constant speed of 12 rotations per minute (rpm). Twenty-four hours prior to drug testing, animals were tested for rotarod performance. Animals that remained on the rotating bar for the full 60 s for 3 consecutive trials were used for the subsequent experiments. The selected animals were randomly distributed into groups of 5 mice and orally received corn oil (control) or trans-caryophyllene (20, 40 and 80 mg/kg). Rotarod tests were performed prior to drug administration (basal) and at 30, 60 and 120 min after administration. The length of time, up to 60 s, that each animal remained on the bar was recorded (Marques et al., 2004).

Acute pain tests

Hot plate

Groups of 5 mice received corn oil (control, p.o.) or trans-caryophyllene (1, 5 or 10 mg/kg, p.o.). An additional group received morphine (5 mg/kg, i.p.) and served as a positive control. After trans-caryophyllene administration, animals were placed on a hot plate (Ugo Basile, Biological Research Apparatus Company, Comerio, Italy) heated to 50 °C. The reaction time was defined as the latency for the animal to lick its paw(s) or jump from the plate. The maximum exposure time was 60 s (Hargraves and Hentall, 2005). Animals were submitted to testing 1, 2, 3 and 4 h after drug administration to verify the duration of the antinociceptive effects.

Formalin test

Groups of 5 mice received corn oil (control, p.o.) or trans-caryophyllene (1, 5 or 10 mg/kg, p.o.). An additional group received morphine (5 mg/kg, i.p.) and served as a positive control. After the drug or control treatment, each animal received an intraplantar injection of 2% formalin (20 μl/animal) into the right paw. Total time spent licking the injected paw was recorded during 2 phases: 5–10 min after injection and 15–30 min after injection (Hunskaar and Hole, 1987).

Mechanism of action (acute pain)

Animals were pretreated with naloxone (1 mg/kg, i.p.) or AM630 (1 mg/kg, i.p.) 30 min before receiving 10 mg/kg of trans-caryophyllene or corn oil (control) to evaluate the participation of the opioid and endocannabinoid systems in the acute antinociceptive activity of trans-caryophyllene. Two additional groups treated with morphine (5 mg/kg, i.p.) and JWH-015 (CB_2 agonist, 1 mg/kg, i.p.) served as positive controls. Thirty minutes after the treatment with the specific agonists or 1 h after the treatment with trans-caryophyllene or corn oil animals were subjected to the hot plate test, as described above.

Chronic pain tests

Induction of neuropathic pain

To induce neuropathic pain, mice were anesthetized (with a combination of xylazine and ketamine, 10 mg/kg, i.p.), and a constriction chronic injury of the sciatic nerve (CCI) was performed according as described by Bennett and Xie (1988) and modified for mice (Martucci et al. 2008). Briefly, the nerve was exposed in the mid-region of the hind limb and close to its trifurcation, was constricted with 4 loose silk thread ligatures (8-0). Sham-operated animals (with the sciatic nerve exposed but no ligature) were used as controls.

Mechanical hypernociception (von Frey test)

Groups of 5 mice were treated with corn oil (control and sham-operated), pregabalin (20 mg/kg, positive control, p.o.) or trans-caryophyllene (1, 5 or 10 mg/kg, p.o.) for 14 days after surgery. The animals were evaluated for tactile stimulation by a von Frey electronic device before surgery (baseline values) and at the 7th and 14th days post-surgery. For this test, a linear crescent pressure was applied in the center of the hindpaw plantar surface until the animal exhibited a characteristic "flinch" withdrawal or licking response. The intensity of the hypernociceptive response was quantified as the change in pressure applied by subtracting the mean of the 3 values obtained before the surgery from the mean of the three values observed on days 7 and 14 post-surgery (Cunha et al. 2004).

Thermal hypernociception (hot plate test)

Thermal hypernociception was evaluated in treated mice for 14 days post-surgery using the hot plate test. Groups of 5 mice received corn oil (control and sham-operated), pregabalin (20 mg/kg, positive control) or trans-caryophyllene (1, 5 or 10 mg/kg, p.o.) for a period of 14 days after surgery. After 1 h of treatment, the animals were placed on a hot plate heated to 50 °C. The reaction time was defined as the latency for the animal to lick its paw(s) or jump from the plate, with a maximum exposure of 60 s. The test occurred before surgery (baseline values) and on the 7th and 14th postoperative days (Hargraves and Hentall, 2005).

Mechanism of action (chronic pain)

To verify the involvement of the opioid and endocannabinoid systems on the antihypernociceptive activity of trans-caryophyllene, animals were treated with corn oil (control surgery) or trans-caryophyllene (10 mg/kg) for 14 days. On the 14th day of treatment, the animals were pretreated with naloxone or AM630 at a dose of 1 mg/kg (i.p.) 30 min before receiving the trans-caryophyllene. The CB_2 agonist-treated group (JWH-015, 1 mg/kg, i.p.), was used as positive control. After 30 min of JWH-015 treatment and 1 h after trans-caryophyllene or corn oil treatment, animals were evaluated using the hot plate test as described above.

Biochemical assay

Enzyme-linked immunosorbent assay (ELISA) tissue sample preparation

After the behavioral tests evaluating neuropathic pain, the IL-1β levels were measured (Safieh-Garabedian et al. 1995). The animals were euthanized, and the sciatic nerve was collected and homogenized in phosphate buffered saline (PBS) containing 0.4 M NaCl, 0.05% Tween 20, 0.5% bovine serum albumin (BSA), 0.1 mM phenylmethyl sulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM