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ORIGINAL ARTICLE

α-Terpineol reduces nociceptive behavior in mice

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Abstract

Context: α-Terpineol (TPN) is a monoterpenoid alcohol present in the essential oils of several species of the *Eucalyptus* genus (Myrtaceae).

Objective: TPN was assessed for its antinociceptive activity in rodents.

Materials and methods: The antinociceptive effect of TPN was examined using the acetic acid writhing reflex, formalin, glutamate, and capsaicin-induced nociception tests.

Results: TPN produced a significant ($P < 0.01$ or $P < 0.001$) analgesic effect by reduction at the early and late phases of paw licking and reduced the writhing reflex in mice (formalin and writhing tests, respectively). In the glutamate test, all doses of TPN produced significant ($P < 0.01$) nociceptive protection. When the capsaicin-induced nociception test was conducted, TPN produced dose-related inhibition of the nociceptive behavior. In addition, the results of a hot plate test showed central analgesic properties for TPN ($P < 0.01$ or $P < 0.001$). Such results were unlikely to be provoked by motor abnormality.

Conclusion: Our results suggest that TPN might represent an important tool for management and/or treatment of painful conditions.

Keywords: monoterpene, α-terpineol, pain, formalin, glutamate, capsaicin

Introduction

Monoterpenes are the main chemical constituents of the essential oils of some medicinal herbs. They are mixtures of odoriferous components obtained by steam distillation or solvent extraction from a large variety of aromatic and medicinal plants (Silva et al., 2009). Besides, monoterpenes and their derivative compounds exhibit several types of pharmacological properties, such as anxiolytic (Silva et al., 2007), antinociceptive (Melo et al., 2010; Quintans-Junior et al., 2010), and anticonvulsant (De Sousa et al., 2006, 2007).

α-Terpineol (TPN), a monoterpenoid alcohol, is a component of the essential oils of several species of plants (Dagne et al., 2000). This compound is widely used in the perfumery, cosmetic, and soap industries (De Sousa et al., 2007). Our group demonstrates that acute treatment with TPN inhibits convulsion induced by pentylenetetrazole and maximal electroshock (De Sousa et al., 2007). Additionally, Golshani et al. (2004) demonstrates that essential oil of *Dracocephalum kotschyi* Boiss (Labiatae), rich in limonene, verbenone, α-terpineol, perillyl alcohol, and caryophyllene, possesses antinociceptive properties. However, no data exist about the possible antinociceptive activity of this isolated compound. Thus, the current investigation was carried out to evaluate the antinociceptive effect of TPN in rodents.

Material and methods

Chemical

Acetic acid and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma (USA).

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Morphine, diazepam, and aspirin were purchased from União Química (Brazil). α-Terpineol (98% purity) was purchased from Sigma (USA).

Animals

Adult male albino Swiss mice (30–35 g) were randomly housed in appropriate cages at 21 ± 2°C with a 12/12-h light/dark cycle (light from 06:00 to 18:00), with free access to food (Purina, Brazil) and tap water. We used 6–8 animals in each group. Nociceptive tests were carried out by the same visual observer and all efforts were made to minimize the number of animals used as well as any discomfort. Experimental protocols were approved by the Animal Care and Use Committee (CEPA/UFS # 26/09) at the Federal University of Sergipe.

Acetic acid writhing reflex

This study was performed according to Koster et al. (1959). Mice (eight per group) were pretreated with TPN (25, 50, and 100 mg/kg), acetylsalicylic acid (aspirin 200 mg/kg), and the vehicle (saline + Tween-80 0.2%) by intraperitoneal (i.p.) route. Then, after 0.5 h, the mice received the 0.85% acetic acid injection (i.p.). The writhing was counted for 15 min after a latency period of 5 min.

Formalin-induced nociception

The procedure described by Hunskaar and Hole (1987) was used. Nociception was induced by injecting 20 µl of 1% formalin in distilled water in the subplantar of the right hind paw. Mice (eight per group) previously received TPN (25, 50, and 100 mg/kg, i.p.), aspirin (200 mg/kg), and vehicle 0.5 h prior to injecting formalin. These mice were individually placed in a transparent plexiglass cage observation chamber (25 cm × 15 cm × 15 cm). The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0 to 5 min (early phase) and 15–30 min (late phase) was counted after injection of formalin (Hunskaar & Hole, 1987).

Glutamate- and capsaicin-induced nociception

The method used was similar to that described previously (De Souza et al., 2009). The animals were placed individually for 5 min in a transparent plexiglass cage observation chamber (25 cm × 15 cm × 15 cm) as an adaptation period. After that, 20 µmol glutamate per paw or 20 µl of capsaicin (1.6 µg/paw prepared in a phosphate-buffered solution) was injected under the skin of the dorsal surface on the right hind paw. The mice were pretreated with TPN i.p. (25, 50, and 100 mg/kg) or morphine (MOR, 3 mg/kg) 0.5 h before injection of the irritant agent (glutamate or capsaicin). The control animals received a similar volume of vehicle (10 ml/kg, i.p.). The amount of time spent licking the injected paw was timed using a chronometer and was considered indicative of nociception.

Hot plate test

The hot plate test described by Jacob and Ramabadran (1978) was used. The animals were placed on an aluminum plate that was adapted to a water bath at 50 ± 0.5°C. The reaction time was noted by observing either the licking of the hind paws at basal, 0.5, 1.0, 1.5, and 2.0 h after i.p. administration of 25, 50, and 100 mg/kg of TPN or the vehicle (saline + Tween-80 0.2%) to different groups of 10 mice. Morphine, 5 mg/kg (i.p.), was used as the reference drug. The effect of pretreatment with naloxone (0.5 mg/kg, i.p.) on the antinociception produced by CTL (100 mg/kg) and morphine (5 mg/kg, i.p.) was determined.

Evaluation of the motor activity

Initially, the mice able to remain on the Rota-rod apparatus (AVS®, Brazil) longer than 180 sec (7 rpm) were selected 24 h before the test (Rosland et al., 1990). Then, the selected animals were divided into five groups (n = 8, per group) and treated, i.p., with vehicle (control), TPN (25, 50, and 100 mg/kg), and diazepam (1.5 mg/kg). Each animal was tested on the Rota-rod and the time (sec) they remained on the bar for up to 180 sec was recorded after 30, 60, and 120 min.

Statistical analysis

The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. In all cases, differences were considered significant if $P < 0.05$.

Table 1. Effect of α-terpineol (TPN) or aspirin on writhing induced by acetic acid and formalin-induced nociception tests.

		Writhing test	Formalin test	
Treatment	Dose (mg/kg)	Number of writhings[a]	0–5 min[a]	15–30 min[a]
Vehicle	—	15.2 ± 3.0	100.5 ± 9.7	126.3 ± 30.1
TPN	25	4.5 ± 1.3[b]	46.0 ± 9.2[b]	17.3 ± 8.9[b]
TPN	50	1.5 ± 0.7[b]	49.1 ± 4.9[b]	0.4 ± 0.3[b]
TPN	100	0.7 ± 0.4[b]	40.5 ± 6.2[b]	14.1 ± 10.3[b]
Aspirin	200	1.9 ± 1.4[b]	83.7 ± 28.9	3.4 ± 2.8[b]

n = 8 per group.
[a]Values represent mean ± SEM.
[b]$P < 0.001$ (one-way ANOVA and Tukey's test), significantly different from control group.

Table 2. Effect of α-terpineol (TPN) or morphine (MOR) on glutamate and capsaicin-induced nociception tests.

Treatment	Dose (mg/kg)	Glutamate test[a]	Capsaicin test[a]
Vehicle	—	58.1 ± 4.5	53.3 ± 0.9
TPN	25	32.5 ± 5.8[b]	25.5 ± 4.4[c]
TPN	50	32.1 ± 5.2[b]	13.2 ± 2.7[c,d]
TPN	100	33.0 ± 3.4[b]	4.5 ± 1.0[c,e]
MOR	5	4.3 ± 2.2c	1.9 ± 1.7[c]

n = 8 per group.
[a]Values represent mean ± SEM.
[b]$P < 0.01$ (one-way ANOVA and Tukey's test), significantly different from control group.
[c]$P < 0.001$ (one-way ANOVA and Tukey's test), significantly different from control group.
[d]$P < 0.05$ (one-way ANOVA and Tukey's test), significantly different from TPN 25 mg/kg group.
[e]$P < 0.05$ (one-way ANOVA and Tukey's test), significantly different from TPN 50 mg/kg group.

Table 3. Antinociceptive effect of α-terpineol (TPN) or morphine (MOR) on the hot plate test.

		Reaction time (licking of the hind paws) (s)[a]				
Treatment	Dose (mg/kg)	Basal	0.5 h	1 h	1.5 h	2 h
Vehicle	—	7.1 ± 2.8	11.5 ± 1.5	9.0 ± 1.4	6.7 ± 1.3	5.7 ± 0.3
TPN	25	6.7 ± 2.5	8.3 ± 0.9	11.8 ± 1.1	9.0 ± 1.2	8.8 ± 1.3
TPN	50	7.9 ± 3.1	11.8 ± 1.2	12.2 ± 1.6	12.2 ± 1.8	9.3 ± 0.8
TPN	100	8.7 ± 2.7	10.5 ± 1.1	16.2 ± 1.6[b]	17.2 ± 2.1[c]	16.7 ± 3.2[c]
MOR	5	7.3 ± 3.2	23.5 ± 2.3[c]	28.1 ± 0.9[c]	27.2 ± 3.6[c]	25.0 ± 3.8[c]

$n = 8$ per group.
[a]Values represent mean ± SEM.
[b]$P < 0.01$ (one-way ANOVA and Tukey's test), significantly different from control group.
[c]$P < 0.001$ (one-way ANOVA and Tukey's test), significantly different from control group.

Results and discussion

The results of this study revealed that TPN has both peripheral and central analgesic properties. The TPN (all doses; i.p.) administered significantly inhibited ($P < 0.001$) the acetic acid-induced writhings and two phases of formalin-induced nociception in mice (Table 1). Those effects are probably in relationship to the inhibition in the peritoneal fluid levels of PGE_2 and $PGF_{2\alpha}$ (Deraedt et al., 1980) and with the release inhibition of substance P, and other inflammatory molecules, such as serotonin, histamine, bradykinin, and prostaglandins (Tjølsen et al., 1992), respectively.

It is well established that glutamate is involved in the transmission of nociceptive signals from the peripheral nervous system to the dorsal horn of the spinal cord. Moreover, it has been reported that glutamate injection evoked pronounced nociceptive responses, which are mediated by neuropeptides (Substance P) releasing from C fibers and due to activation of glutamate receptors [i.e., *N*-methyl-D-aspartic acid (NMDA)] that can stimulate the production of a variety of intracellular second messengers, such as NO, and proinflammatory cytokines as tumor necrosis factor-alpha (TNF-α) and IL-1β, which act synergistically in the excitation of the neurons (Ribas et al., 2008). Additionally, Beirith et al. (2002) have found that the nociceptive response induced by glutamate appears to involve peripheral, spinal, and supraspinal sites of action and is greatly mediated by both NMDA and non-NMDA receptors. TPN produced an inhibition of the nociception induced by glutamate (Table 2).

Sakurada et al. (2003) proposed the capsaicin-induced pain model for the study of compounds that act on pain of neurogenic origin. Capsaicin is a neurotoxic compound extracted from red pepper which, when applied to the skin or injected into animals, produces irritation, a painful reaction, and subsequent desensitization to chemically induced pain. Studies have shown that capsaicin evokes the release of neuropeptides, excitatory amino acids (glutamate and aspartate), nitric oxide (NO), and proinflammatory mediators in the periphery, and transmits nociceptive information to the spinal cord (Duarte et al., 1988). Waning et al. (2007) demonstrated that the capsaicin-sensitive transient receptor potential vaniloid 1 (TRPV1), which plays an important role in pain transduction, is one of the Ca^{+2} influx channels involved in cell migration. Our results indicate a significant reduction in neurogenic nociception caused by the intraplantar injection of capsaicin, showing that TPN caused significant effects in this test. The capsaicin-induced neurogenic paw licking response was similar to the first phase of the formalin test at TPN (all doses).

The analgesic action presented by TPN involves supraspinal as well as spinal components, as demonstrated by the utilization of the hot plate test (Le Bars et al., 2001). The results suggest that TPN (at only the higher dose) has a central analgesic effect, as evidenced by the prolonged delay in response time when mice were subjected to a nociceptive stimulus during a hot plate test (Table 3).

To investigate whether the treatments with TPN could influence the motor activity of the animals and consequently impair the assessment of the nociceptive behavior in the experimental models, the motor activity of the animals was evaluated with a Rota-rod apparatus (Quintans-Júnior et al., 2010). TPN treated mice did not show any significant motor performance alterations with the doses of 25, 50, or 100 mg/kg (data not shown).

Together, these results indicate that TPN, an important monoterpene of the Eucalyptus species, might represent an important tool for treatment of pain conditions. Further studies currently in progress will enable us to understand the precise action mechanisms.

Declaration of interest

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