Biomedicine & Pharmacotherapy 105 (2018) 652–661

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

α-Terpineol reduces cancer pain via modulation of oxidative stress and inhibition of iNOS

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ARTICLE INFO

Keywords:
Monoterpenes
α-Terpineol
Oxidative stress
Oncologic pain

ABSTRACT

α-Terpineol (TP) is present in a wide range of essential oils of the genus *Eucalyptus*, with recognized potential for a range of biological effects, such as analgesic. Hence, our study aimed to investigate the effect of TP on cancer pain induced by sarcoma 180 in *Swiss* mice. Our results showed that TP reduced significantly mechanical hyperalgesia and spontaneous and palpation-induced nociception, improved paw use without reducing tumor growth and grip strength. Importantly, no evident biochemical and hematological toxicity was oberved. Furthermore, TP increased the tissue antioxidant capacity due to ferric-reducing antioxidant power (FRAP) and glutathione (GSH). TP also reduced inducible nitric oxide synthase (iNOS) immunocontent in the tumors. Molecular docking estimated that TP binds within the same range of iNOS regions (other iNOS inhibitors), such as N-Nitroarginine methyl ester (L-NAME). These data provide strong evidence that TP may be an interesting candidate for the development of new safe analgesic drugs that are effective for cancer pain control.

1. Introduction

The prevalence of cancer is increasing globally, with 17 million new cases predicted for 2020, whereby pain is one of the most prevalent, costly and distressing symptoms experienced by cancer patients [1]. Oncologic pain affects 75–90% of patients with advanced disease stage and can be considered as a significant factor for life quality impairment [2,3].

Oncologic pain presents a complex and multifactorial neurobiology. The excessive proliferative status of cancer cells is considered as a fundamental property of cancer [4]. Besides, the external microenvironment that surrounds the cancer cells can also be altered by the activation of the immune system, with the recruitment of macrophages, neutrophils and T-cells and the consequent production of inflammatory mediators [5]. Inflammation is often accompanied by increased reactive species, revealing that the oxidative microenvironment surrounding tumor cells is highly associated with increased oxidative stress, either directly by cancer cells or indirectly by the activation of the immune system [6].

Because of its pathophysiological complexity, up to 15% of nearly 7 million chronic pain patients can not be completely relieved by the conventional management of this symptom. That occurs largely due to the side effects associated with the treatment such as constipation, nausea, vomiting, sedation, respiratory depression, dependence, tolerance, bleeding, gastrointestinal ulceration, renal toxicity, hypotension, cardiotoxicity, among others [5,6]. In this sense, a systematic review has showed that medicinal plants have historically proved their therapeutic potential and, today, still stand out as an important grouping for the identification of new analgesic drugs [7,8].

α-terpineol (TP) is an alcoholic monoterpene found in the essential oil of several species belonging to the genus *Eucalyptus* [9]. This monoterpene has peripheral-mediated antinociceptive effect due to its ability to inhibit the cyclooxygenase enzyme and the production of inflammatory mediators and cytokines such as prostaglandin E2

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https://doi.org/10.1016/j.biopha.2018.06.027
Received 2 March 2018; Received in revised form 5 June 2018; Accepted 5 June 2018

(PGE_2), interleukin-1β (IL-1β) and nuclear factor kappa B (NF-kB) [10–15]. This substance also modulates the levels of nitric oxide (NO), an important marker of oxidative stress [16]. However, studies that show the effect of TP on chronic pain, such as cancer pain, are scarce.

Considering the need for new therapeutic options for oncologic pain and the therapeutic potential of monoterpenes, especially TP, this study aimed to evaluate the effect of TP on nociceptive responses induced by sarcoma 180 (S180) in rodents.

2. Methods

2.1. Chemicals

α-Terpineol (96% purity), cremophor, sodium chloride, tripan blue, nitrobenzoic acid, glycerol, β-mercaptoethanol, bromophenol blue, tween, thiobarbituric acid, Tetraethoxypropane (TEP), tripyridyltriazine (TPTZ), dinitrobenzoic acid (DTNB) and glutathione (GSH) were purchased from Sigma (USA). Morphine and lactated ringer's solution were purchased from Cristália (São Paulo, São Paulo, Brazil). Ketamine and Xylazine were purchased from Cristália (Itabira-SP, Brazil). Acetic acid, hematoxylin and eosin were purchased from Synth and EDTA from Neon. Protease inhibitors were derived from Sigma (FAST), primary anti-iNOS and anti-β-actin antibodies (Santa Cruz Biotechnology Inc - Santa Cruz, CA, USA). Anti-rabbit IgG-HRP and IgG-HRP anti-mouse secondary antibodies were purchased from Sigma (St. Louis, MO).

2.2. Animals

Male Swiss mice used (28–32 g; 2–3 months of age) were randomly housed in appropriate cages at 22 ± 2 °C on a 12 h light/dark cycle with free access to food (Purina®, Brazil) and water. Experimental protocols were approved by the Animal Care and Use Committee (CEPA/UFS 05/14) at the Universidade Federal de Sergipe, and all handling procedures were in accordance with the International Association for the Study of Pain (IASP) guidelines for the use of animals in pain research.

2.3. Tumor cell and implantation

S180 tumor cells that had been maintained in the peritoneal cavity of Swiss mice were obtained from the Laboratory of Clinical and Experimental Oncology at the Federal University of Sergipe. A suspension of 10^6 viable S180 cells per 25 µl of lactated Ringer's solution was implanted subcutaneously into the plantar region of mice. Animals of the sham group received only 25 µl of lactated Ringer's solution [17,18].

2.4. Treatment

Twenty-four hours after administration of S180, animals (n = 08/group) were treated daily with vehicle (saline + cremophor 0.4% v/v), TP (12.5, 25 or 50 mg/kg) or morphine (15 mg/kg) via subcutaneous route until the fifteenth day and were then submitted to behavioral evaluation on alternate days. The route of administration of the treatments was subcutaneous so that the hepatic first-pass effect was avoided. The animals were randomly distributed between the groups and the evaluations were performed blindly in order to reduce the assessment bias.

2.5. Behavioral studies

The mechanical hyperalgesia was assessed by means of digital von Frey (Model: EFF-301, Insight®, Brazil) through hind paw flexion reflex, which corresponds to the paw withdrawal followed by clear flinching movements. In order to evaluate the spontaneous nociception, mice were placed scattered in boxes and allowed to acclimate for 10 min. Afterwards, the flinching behaviors were observated during a 10-min period. Non-noxious palpation of the tumor-bearing paw was performed during 2 min and the number of flinching behaviors was quantified for 2 min to determine the palpation-induced nociception [19]. The use of the limb was evaluated as previously described by Luger et al. [20], through the observation of the mouse while walking in a continuous movement. The limb and/or guard behavior of the right hind limb (treated with sarcoma) was evaluated in the following scale: 0 = complete lack of use, 1 = partial limb use in locomotor activity, 2 = limb and guard behavior, 3 = substantial limping and 4 = normal walking [20].

2.6. Measurement of forelimb grip strength

In order to check for possible changes in neuromuscular function, such as the myorrelaxant effect, we measured the tension force of limbs using the commercial grip strength meter (Insight®, Brazil) before the treatment (s.c.) of tumor-free animals with vehicle or TP (12.5, 25 or 50 mg/kg) and 30, 60 and 120 min after treatment [21].

2.7. Measurement of paw volume

The effect of TP on tumor growth of S180 was evaluated through right paw volume, which was measured using plethysmometer (Insight®, Brazil) before (time zero) and on every other day up to 15 days.

2.8. Toxicity

Toxicity assessment was performed by means of the weight control of the animals and behavioral changes prior to tumor inoculation daily until the 15th day of the experiment. On the last day, the biochemical and hematological analyses were performed to evaluate glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, urea, creatinine, the total and differential leukocyte count, red blood cells, hemoglobin, hematocrit and platelets. The weights of vital organs such as heart, lung, brain, liver and kidneys were checked after the euthanasia of the mice, which were macroscopically observed. The relative weight of the organs was calculated as follows: Relative weight = [(body weight/body weight) x 100]. After that, the organs were submitted to histopathological analysis after staining with hematoxylin and eosin. Finally, the slides were analyzed in an optical microscope and documented in a photo microscope (Olympus®) using standard and polarized polychromatic light.

2.9. Histology

Different groups of animals were euthanized with excessive sedation on the 15th day after sarcoma inoculation. The paws were then submitted to fixation with 10% formalin solution, decalcified in 10% EDTA (pH 7.4) for two weeks and submitted to conventional histological processing. Sections were cut in the sagittal plane and stained with hematoxylin and eosin for optical microscopic visualization of the histopathological characteristics of the tumor [22].

2.10. Antioxidant capacity of tissues

On the 15th day, tumor, spinal cord and brain were homogenized using turrax-type homogenizer in ice bath and 0.1 M potassium phosphate buffer pH 7.0. The homogenate was centrifuged at 15,000 rpm for 30 min at 4 °C. The supernatant was used for the determination of reducing power (FRAP), glutathione (GSH) and thiobarbituric acid reactive substances (TBARS).

For the FRAP assay, in a 96-well plate, a 9-µl aliquot of the homogenate was mixed with 27 µL of distilled water and 270 µL of the FRAP