Fitoterapia 129 (2018) 20–24

Contents lists available at ScienceDirect

Fitoterapia

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

Antinociceptive effect of inhalation of the essential oil of bergamot in mice

Damiana Scuteri[a], Michele Crudo[a], Laura Rombolà[a], Chizuko Watanabe[b], Hirokazu Mizoguchi[b], Shinobu Sakurada[b], Tsukasa Sakurada[c], Rosaria Greco[d], Maria Tiziana Corasaniti[e], Luigi Antonio Morrone[a], Giacinto Bagetta[a,*]

[a] Preclinical and Translational Pharmacology, Department of Pharmacy, Health Science and Nutrition, University of Calabria, 87036 Rende, CS, Italy
[b] Department of Physiology and Anatomy, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan
[c] Daiichi College of Pharmaceutical Sciences - First Department of Pharmacology Fukuoka, Japan
[d] Laboratory of Neurophysiology of Integrative Autonomic Systems, Headache Science Centre, IRCCS Mondino Foundation, Pavia, Italy
[e] Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro, Italy

ARTICLE INFO

Keywords:
Aromatherapy
Bergamot essential oil
Pain
Inhalatory route
BPSDs

ABSTRACT

Bergamot essential oil (BEO) has proven wide evidence of pharmacological antinociceptive effectiveness both in nociceptive and in neuropathic pain models. The antinociceptive properties of BEO for inhalation have not been investigated. The purpose of this study is to evaluate the effects of the inhalation of BEO on formalin-induced nociceptive response in mice.

Male ddY-strain mice (Japan SLC, Hamamatsu, Japan) of 23–25 g of weight at the time the experiments underwent the formalin test. Twenty μl of formalin (2% in saline) were administered into the plantar surface of the mice hindpaw and the time of licking/biting was observed and recorded at intervals of 5 min. The device for BEO inhalatory delivery consisted in a filter paper disc soaked with known volume of BEO placed on the edge of the cage.

Inhalation of BEO exerted antinociceptive activity. In particular, it reduced the formalin-induced licking/biting behaviour in a manner that was dependent on the volume of BEO used in the device for its release and on the time of exposure to the phytocomplex.

The results support the use of BEO in aromatherapy for complementary management of chronic pain relief in a stepwise therapeutic programme.

1. Introduction

According to the Farmacopea Ufficiale Italiana (1991) bergamot essential oil (BEO) is obtained by cold pressing of the epicarp and, partly, of the mesocarp of the fresh fruit of bergamot (*Citrus bergamia* Risso et Poiteau). BEO comprises a volatile fraction (93–96% of total) containing monoterpene and sesquiterpene hydrocarbons (such as limonene) and oxygenated derivatives (such as linalool) and a non-volatile fraction (4–7% of total) containing waxes, polymethoxylated flavones, coumarins and psoralens such as bergapten (5-methoxypsoralen) and bergamottine (5-geranyloxypsoralen) [1, 2]. The most abundant compounds found in the volatile fraction are the monoterpene hydrocarbons limonene, γ-terpinene, and β-pinene, the monoterpene alcohol, linalool, and the monoterpene ester, linalyl acetate, which altogether constitute > 90% of the whole oil [3–5]. The nonvolatile residue is a natural odor fixative which influences the olfactory properties of the oil; however, it contains about 0.2% bergapten which is responsible for the phototoxicity of BEO [6, 7]. Therefore, a bergapten-free extract of the essence (BEO-BF) together with a natural essence deprived of the hydrocarbon fraction and of bergapten (BEO-HF/BF) are prepared by extractive industries for perfumery and cosmetic uses. Recently, this essential oil has been rigorously studied and some pharmacological activities of the utmost importance have been deciphered. In particular, strong evidence has been gathered for BEO to be endowed with analgesic activity, both in nociceptive and in neuropathic pain models (see [8]). In fact, intraplantar (i.pl.) BEO, or its components linalool and linalyl acetate, reduced the nociceptive response as assayed by the capsaicin test [9]. The latter antinociceptive effects were antagonized by the ipsilateral i.pl. injection of naloxone hydrochloride and by intraperitoneal (i.p.) naloxone methiodide, an

Abbreviations: BPSDs, Behavioural and psychological symptoms of dementia; BEO, Bergamot Essential Oil; BEO-BF, Bergapten-free extract of the essence; BEO-HF/BF, Essence deprived of the hydrocarbon fraction and of bergapten; i.p., Intraperitoneal; i.pl., Intraplantar; i.t., Intrathecal; PSNL, Partial Sciatic Nerve Ligation

* Corresponding author.
E-mail address: g.bagetta@unical.it (G. Bagetta).

https://doi.org/10.1016/j.fitote.2018.06.007
Received 14 May 2018; Received in revised form 9 June 2018; Accepted 11 June 2018
Available online 12 June 2018
0367-326X/

antagonist acting at the peripheral opioid receptors; morphine-induced antinociception after i.p. and intrathecal (i.t.) injections was markedly enhanced by the combined injection of i.pl. BEO or linalool, its main oxygenated monoterpene [9]. Interestingly, for i.pl. injection BEO or linalool reduced partial sciatic nerve ligation (PSNL)-induced neuropathic pain symptoms in mice and inhibition of spinal ERK phosphorylation seems to be involved in this anti-allodynic effect [10]. These same Authors [9] have shown that BEO or linalool modulate morphine-induced anti-allodynic effect under neuropathic pain, a condition known to be resistant to opioid treatment (see [11]). More recently, for i.pl. injection BEO and linalool have been reported to reduce behavioural signs of formalin-induced nociception in a dose-dependent manner. The formalin test is characterized by formalin-induced biphasic nocifensive behaviour of licking/biting, with the early nociceptive phase being followed by a late, second, phase that involves peripheral inflammation and central sensitization (see [12]). Due to the lack of relevant information about the effects of the inhalation of BEO on nocifensive behaviour, the purpose of this study was to investigate the antinociceptive action of BEO *via* the inhalatory route of administration. Indeed, according to the literature, BEO inhalation was found to produce anxiolytic-like behaviour [13], but there are no data available about its effect on nociception and this may be relevant to the use of BEO in aromatherapy.

2. Materials and methods

2.1. Animals

For the study, male ddY-strain mice (Japan SLC, Hamamatsu, Japan) of 23–25 g of weight at the time of these experiments were used. Mice were individually housed in a colony maintained in a controlled environment (12 h light/dark cycle, room temperature 23 °C, 50–60% relative humidity), with food and water *ad libitum*. All of the experiments were performed in agreement with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals and approved by the Committee of Animal Care and Use of Tohoku Medical and Pharmaceutical University, for minimizing animal suffering and to use only the number of animals necessary to produce reliable results.

2.2. Experimental protocol for BEO administration via inhalatory route

The habituation of mice was carried out in a transparent cage (22.0 cm × 15.0 cm × 12.5 cm), that also served as observation chamber. Another plexiglass cage was turned upside down and placed over the first cage, in order to avoid any leaks of BEO. A filter paper dry disc (control) or soaked with different volumes of BEO according to the experiment (100, 200, 400, 800 µl) was applied on the edge of the cage 5 min before placing the mice in the observation chamber, so that it was saturated with BEO. Mice were divided into three experimental groups (post-inhalation, pre-inhalation and double-inhalation). In the post-inhalation group the inhalation of BEO was carried out immediately after the i.pl. injection of formalin and for the whole duration of the formalin test. In the pre-inhalation group the inhalation of BEO was carried out as pre-treatment for 1 h, during the mice habituation, at the end of which BEO-releasing filter paper was removed and the formalin test was performed. In the double-inhalation group the inhalation of BEO was carried out both as pre-treatment for 1 h during the habituation and immediately after formalin administration for the whole duration of the formalin test, in order to assess the total effects of these two different options of delivery of BEO.

2.3. Formalin test

After 1 h of habituation 20 µl of formalin (2% in saline) were i.pl. administered to the mice, using a microsyringe with 26-gauge needle. The time of licking/biting was recorded with a handheld stop-watch at intervals of 5 min: during the early phase, beginning immediately after formalin administration and lasting for 10 min (0–10 min), and during the late phase, starting 10 min after formalin injection and lasting for 20 min (10–30 min).

2.4. BEO composition

BEO was obtained from “Capua Company1880 S.r.l.,” Campo Calabro, Reggio Calabria (Italy). According to chromatographic analysis provided in the certificate of analysis, this batch of BEO contains: D-limonene (39.60%), linalyl acetate (31.09%), linalool (9.55%).

2.5. Statistical analysis

The results are presented as mean ± s.e.m. duration (seconds) of nociceptive response and evaluated statistically for differences by ANOVA followed by Bonferroni's test and considered significant when $p < 0.05$.

3. Results

3.1. Effect of BEO inhalation as post-treatment on formalin test evoked licking/biting

In the post-inhalation group (see treatment scheme in Fig. 1 a) the filter paper disc, applied on the edge of the cage, was soaked with 200, 400 or 800 µl of BEO and the mice were subjected to BEO inhalation from the time of formalin injection for the following 30 min, during which the formalin test was carried out. Under these experimental conditions, BEO did not show significant effects on the early phase (0–10 min) (Fig. 2). However, administration of 400 and 800 µl of BEO significantly reduced the time of licking/biting in the late phase (10–30 min) in a dose-dependent manner (Fig. 2).

3.2. Effect of BEO inhalation as pre-treatment on licking/biting

Mice were subjected to the inhalation of BEO (filter paper disc soaked with 200, 400 or 800 µl) for the whole habituation period of 1 h (see treatment scheme in Fig. 1 b) but not during the formalin test.

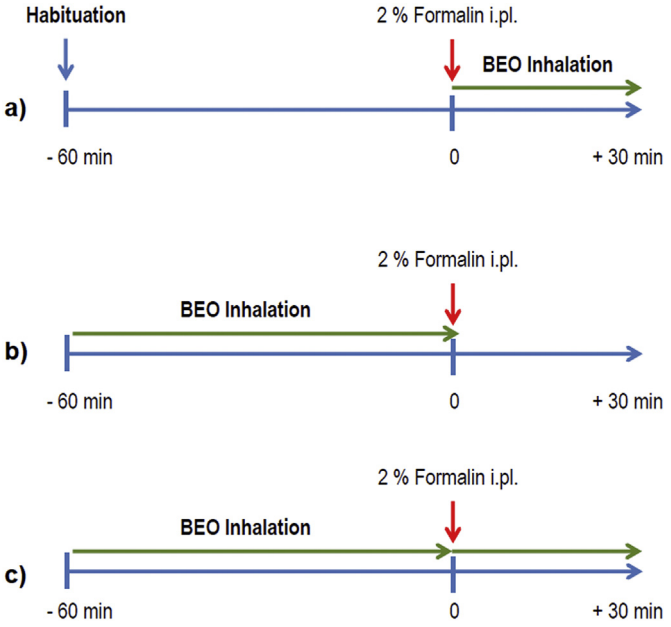

Fig. 1. BEO inhalation scheme. Schematic representation of the administration scheme of BEO as: a) post-inhalation, b) pre-inhalation and c) double-inhalation in relation to formalin intraplantar (i.pl.) administration.