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Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constituent of eucalyptus and rosemary oils

Flávia A. Santos, Vietla S.N. Rao *

Departamento de Fisiologia e Farmacologia, Centro de Ciências da Saúde, Universidade Federal do Ceará, CP 3157, 60430-270 Fortaleza, CE, Brazil

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Abstract

The present studies tested the ability of 1,8-cineole to produce inflammatory oedema in the hind paw of the rat and verified the possible involvement of mast cells in the response. Subplantar injection of 1,8-cineole (10, 15 and 20 μl/paw) induced a dose-dependent paw oedema which was apparent within 30 min. At higher doses the oedema effect was persistent, peaked at 2 h, and then decreased gradually but was still pronounced at 24 h post injection. In contrast, the oedema produced by mast cell degranulator compound 48/80 (10 μg/paw) had a rapid onset with a peak effect at the first hour, followed by a gradual decrease thereafter and at 24 h post injection it was almost absent. The oedema response to 20 μl 1,8-cineole was significantly inhibited throughout its time-course in rats pretreated with antihistaminic and antiserotonergic drugs such as diphenhydramine, methysergide and cyproheptadine or with ketotifen, a mast cell stabilizer. A more effective blockade of the oedema response was, however, observed in rats depleted of mast cell granules by systemic treatment with compound 48/80. Furthermore, 1,8-cineole was able to cause rat peritoneal mast cell degranulation (94%) in vitro, in a concentration as low as 0.3 μl/ml, which was almost comparable to that produced by 0.1 μg/ml of compound 48/80. The data provide evidence of a key role for the mast cell in 1,8-cineole-induced hind paw oedema in the rat. © 1997 Elsevier Science B.V.

Keywords: 1,8-cineole; Paw oedema; Mast cell; Compound 48/80; Ketotifen; Cyproheptadine; (Rat)

1. Introduction

In recent years there has been a tremendous upsurge in the utilization of plant essential oils for aromatherapy, to treat minor gastrointestinal disorders and headaches, and to stimulate the skin and improve circulation (Eichholz, 1948; Weyers and Brodbeck, 1989; Gobel et al., 1995).

Essential oils and their constituents are most commonly applied to the skin or given orally for their therapeutic benefits. They do present risks of skin sensitization and irritation, carcinogenicity and systemic toxicity (Tisserand and Balacs, 1995). Understanding the actions of the principal constituents of essential oils is a formidable task for the pharmacologist when assessing the risks associated with aromatherapy.

1,8-Cineole (cineole) also known as eucalyptol or cajeputol is a terpenoid oxide and is the main constituent of most *Eucalyptus* oils (~ 75%), rosemary (~ 40%), *Psidium* (40–63%) and many other essential oils (Kovar et al., 1987; Andrade Neto et al., 1994; Gobel et al., 1995). Being non-toxic, non-irritant and nonsensitizing (Opdyke, 1976), it is often employed in drug formulations as a percutaneous penetration enhancer and for its decongestant and antitussive effects and in aromatherapy as skin stimulant in the form of skin baths (Macht, 1938; Williams and Barry, 1991; Laude et al., 1994; Levison et al., 1994). Nonfatal serious toxicity symptoms have been observed in children following nasal application of cineole and the effects included irritable mucous membranes, tachycardia, dyspnoea, nausea, vomiting, muscular weakness, drowsiness and coma (Melis et al., 1990). Published reports indicate that the rate of diffusion of 1,8-cineole is also more rapid following inhalation or oral administration than after skin application (Tisserand, 1985; Kovar et al., 1987). Since the stratum corneum serves as a protective barrier for percutaneous absorption, the risk of toxicity is far less, but when the skin is damaged, the rate of percutaneous absorption can greatly increase and may provoke local or systemic reactions. We now provide evidence that 1,8-cineole possesses local irritant properties and induces oedema following subplantar injection into the hind paw of rats. Further, in order to gain an insight into the role of

* Corresponding author. Tel.: (55-85) 243-9341; Fax: (55-85) 243-9333.

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mast cells, we studied the modulatory influence of various pharmacological agents on 1,8-cineole-induced paw oedema.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (150–180 g) were housed in groups of six under environmentally controlled conditions (12 h light–12 h dark cycle; 22 ± 3°C) with free access to water and food for seven days before the experiments. Food was withheld 15 h prior to experimentation whereas water was allowed ad libitum.

2.2. Rat paw oedema

The ability to induce oedema was tested by subplantar injection of 1,8-cineole (10, 15 and 20 μl/paw) into the left hind paw of conscious rats. An equal volume of saline (NaCl, 0.9%) was injected into the contralateral paw. The paw oedema was measured using a plethysmograph (model 7150, Ugo Basile, Milan, Italy) at 30 min, 1, 2, 3, 4 h and 24 h after 1,8-cineole injection. The results were expressed as the difference in volume (ml) of the 1,8-cineole-injected paw and the contralateral paw, which was injected with saline.

2.3. Depletion of histamine and 5-hydroxytryptamine (5-HT)

Rats were depleted of their stores of histamine and 5-HT by repeated injections of compound 48/80, as previously described (Di Rosa et al., 1971). Briefly, a 0.1% (w/v) solution of compound 48/80 in saline was given intraperitoneally twice a day for 4 days. The doses used were 0.6 mg/kg for the first 6 injections and 1.2 mg/kg for the last two doses. 1,8-cineole was injected 5–6 h after the last injection of 48/80.

2.4. Depletion of substance P

Rats were depleted of substance P by subcutaneous injections of Capsaicin (2 × 50 mg/kg), as described (Jessell et al., 1978). The rats were used seven days later to study the influence of substance P depletion on 1,8-cineole-induced paw oedema.

2.5. Mast cell degranulation

Rats were killed with excess ether. Pieces of mesentery free from fat and blood vessels were quickly obtained and placed in Ringer-Locke solution (NaCl 154 mM, KCl 5.6 mM, $CaCl_2$ 1.6 mM, $NaHCO_3$ 1.78 mM and glucose 5.5 mM), as previously described (Norton, 1954), and incubated in solutions of 1,8-cineole (0.1, 0.3 and 1.0 μl/ml), compound 48/80 (0.03, 0.1 and 0.3 μg/ml) or vehicle in Ringer-Locke solution for 30 min at room temperature. Two pieces of mesentery were used for each concentration of drug. Tissues were removed, mounted on glass slides and stained with toluidine blue (0.1%). For each concentration a total number of 50 mast cells were counted from five microscopic fields selected at random under light microscopy, using 430 × magnification, and the percentage of degranulated cells was obtained.

2.6. Drugs

1,8-cineole, Compound 48/80, indomethacin, nordihydroguiretic acid and capsaicin were obtained from Sigma Chemical (St.Louis, MO). The other drugs used were dexamethasone (Decadron^R, M.S.D, Brazil), methysergide (Deserila, Sandoz, Brazil), thalidomide (Thalidomida, CEME, Brazil), cyproheptadine (Periatin^R, M.S.D, Brazil), ketotifen (Ketasma^R, Sun Pharmaceutical, India) and diphenhydramine (Benadril^R, Parke Davis, Brazil). Dexamethasone, indomethacin, diphenhydramine, methysergide, thalidomide and cyproheptadine were suspended in a vehicle consisting of 0.2% carboxymethylcellulose and were administered orally in a volume of 10 ml/kg 1 h before 1,8-cineole, with the exception of dexamethasone, which was administered 2 h before. Compound 48/80 was dissolved in saline solution (0.9% (w/v) NaCl) whereas Capsaicin was suspended in 10% ethanol. 1,8-cineole and nordihydroguiretic acid were suspended in 2% Tween 80.

2.7. Statistical analysis

Results are expressed as the means ± S.E.M. The data on paw oedema were evaluated by a one- and two-way (treatment × time) analysis of variance (ANOVA) with repeated measures, and a post-hoc Newman–Keuls test for multiple comparisons. For mast cell degranulation data, statistical analysis was done by Kruskal–Wallis nonparametric analysis of variance followed by Dunnett's multiple comparison test. P values of 0.05 or less were considered significant.

3. Results

3.1. Time-course of paw oedema induced by 1,8-cineole and compound 48/80

The subplantar injection of 1,8-cineole in doses of 10, 15 and 20 μl/paw produced a dose- and time-dependent oedema (Fig. 1). At a smaller dose (10 μl/paw), the peak oedema noticed at 30 min was gradually diminished

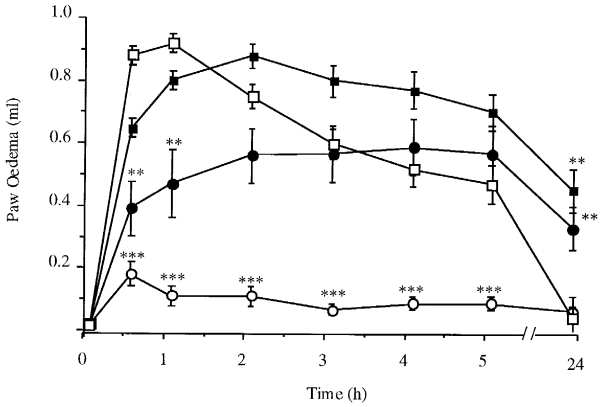

Fig. 1. Time-course of oedema formation induced by subplantar injection of 1,8-cineole, ○ 10 μl; ● 15 μl; ■ 20 μl and □ compound 48/80, 10 μg into the left hind paw of rats. An equal volume of saline was injected into the contralateral paw. The oedema was measured plethysmographically and expressed as the difference in volume (ml) between the drug-injected paw and the contralateral paw, which was injected with saline. Each point represents the mean ± S.E.M. from six animals. $^{**}P < 0.01$, $^{***}P < 0.001$ in comparison with compound 48/80.

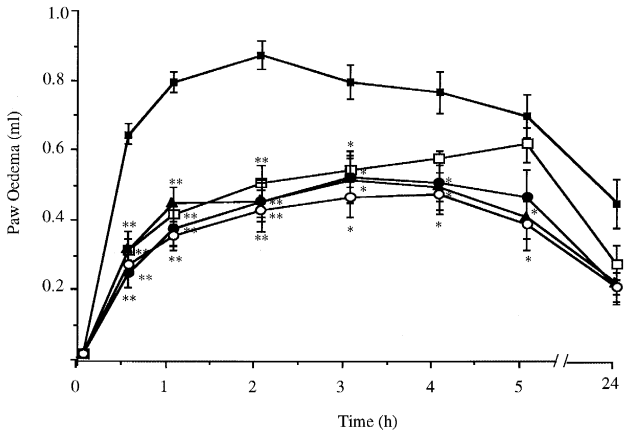

Fig. 2. Time-course of the inhibitory effects of diphenhydramine, methysergide, cyproheptadine and ketotifen on oedema formation induced by 1,8-cineole in the hind paw of rats. ■ Vehicle 10 ml/kg, p.o.; ● diphenhydramine 5 mg/kg, p.o.; □ methysergide 1 mg/kg, p.o.; ○ cyproheptadine 5 mg/kg, p.o.; ▲ ketotifen 1 mg/kg, p.o. All drugs were administered 1 h before subplantar injection of 20 μl 1,8-cineole. Each value represents the mean ± S.E.M. obtained from six animals. $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with vehicle.

whereas at higher doses (15 and 20 μl/paw), 1,8-cineole induced greater oedema, with a peak effect at 2 h and a long duration of action. Paw oedema induced by mast cell degranulator compound 48/80 (10 μg/paw) had a rapid onset, showed its peak effect at 1 h, decreased gradually between 2 and 5 h, and at 24 h post injection it was almost absent. The oedema reaction produced by 20 μl 1,8-cineole was not statistically different from that of compound 48/80 up to 5 h. However, the paw oedema-inducing effect of higher doses of 1,8-cineole (15 and 20 μl) at 24 h post injection was significantly ($P < 0.01$) greater than that of compound 48/80.

3.2. Effects of diphenhydramine, methysergide, cyproheptadine and ketotifen on 1,8-cineole-induced paw oedema

The involvement of 5-HT and/or histamine on the 1,8-cineole-induced oedema was investigated in rats pretreated with diphenhydramine (5 mg/kg), methysergide (1 mg/kg), cyproheptadine (5 mg/kg) or ketotifen (1 mg/kg). Rats treated with cyproheptadine (5-HT and histamine receptor antagonist), diphenhydramine (antihistamine), methysergide (5-HT receptor antagonist) and ketotifen (mast cell stabilizer) showed markedly less oedema than vehicle-treated controls (Fig. 2). The oedema inhibition caused by these agents was highly significant ($P > 0.01$) during the time periods of 30 min, 1 and 2 h. Less significant inhibition ($P < 0.05$) of 1,8-cineole oedema was, however, also observed with cyproheptadine and ketotifen during 3–5 h, with methysergide at 3 h and with diphenhydramine at 3 and 4 h. None of these drugs were able to significantly inhibit 1,8-cineole-induced oedema at 24 h.

3.3. Effects of capsaicin, compound 48 / 80, nordihydroguiretic acid and thalidomide on paw oedema induced by 1,8-cineole

We examined whether depletion of substance P by systemic treatment of rats with capsaicin (2 × 50 mg/kg, s.c.) and depletion of histamine and 5-HT by repeated injections of compound 48/80 (6 × 0.6 mg/kg and 2 × 1.2 mg/kg, i.p.) or treatment with lipoxygenase inhibitor, nordihydroguiretic acid (75 mg/kg, i.p.), or tumor necrosis factor antagonist, thalidomide (15 mg/kg, p.o.), would have any influence on the paw oedema induced by 1,8-cineole.

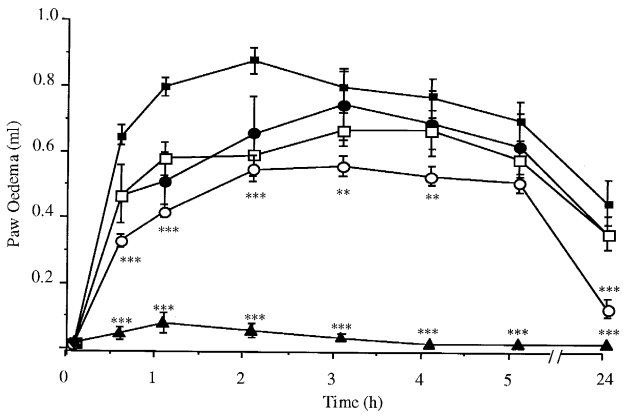

Fig. 3. Effects of depletion of substance P by □ capsaicin (2×50 mg/kg, s.c.), depletion of histamine and 5-HT by ▲ compound 48/80 (6×0.6 mg/kg and 2×1.2 mg/kg, s.c.; twice daily on four consecutive days), lipoxygenase inhibition by ● NDGA (75 mg/kg, i.p.), tumor necrosis factor antagonism by ○ thalidomide (15 mg/kg, p.o.) and ■ vehicle (10 ml/kg, p.o.) on 1,8-cineole (20 μl)-induced hind paw oedema in rats. Each value represents the mean ± S.E.M. obtained from 6 animals. $^{**}P < 0.01$, $^{***}P < 0.001$ in comparison with vehicle.

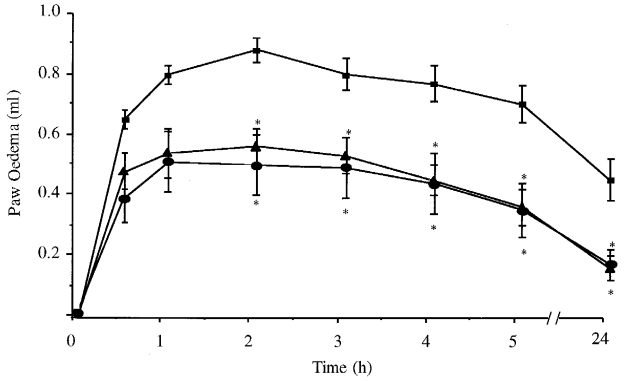

Fig. 4. Time-course of the inhibitory effects of indomethacin and dexamethasone on hind paw oedema induced by subplantar injection of 1,8-cineole (20 μl) in rats. Dexamethasone was injected 2 h before and indomethacin 1 h before 1,8-cineole. ■ vehicle, 10 ml/kg, p.o.; ▲ indomethacin, 2 mg/kg, p.o.; ● dexamethasone, 0.5 mg/kg, p.o. Each value represents the mean ± S.E.M. obtained from 6 animals. $^{*}P < 0.05$ in comparison with vehicle.

Depletion of substance P by capsaicin or lipoxygenase inhibition by NDGA did not show any significant influence on the time-course response curve for oedema induced by the subplantar injection of 1,8-cineole. In contrast, depletion of histamine and 5-HT in rats systemically treated with 48/80 resulted in almost complete inhibition of the paw oedema-inducing effect of 1,8-cineole (Fig. 3). Thalidomide-pretreated rats also showed significantly less oedema at all time points of observation except at 5 h.

3.4. Effects of indomethacin and dexamethasone

Pretreatment of rats with the cyclo-oxygenase inhibitor, indomethacin (2 mg/kg, p.o., 1 h before), or with dexamethasone (0.5 mg/kg, p.o., 2 h before) did not have a significant influence on 1,8-cineole-induced early oedema (30 min and 1 h). However, these agents were able to significantly suppress the oedema response to 1,8-cineole at the time points of 2, 3, 4, 5 and 24 h (Fig. 4).

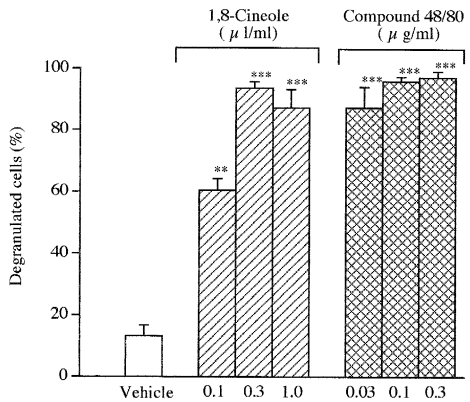

Fig. 5. Effects of 1,8-cineole, compound 48/80 or the vehicle on rat peritoneal mast cell degranulation in vitro. The drugs and/or vehicle were added to the nutrient solution (Ringer-Locke) containing pieces of mesentery and incubated for 30 min. The values are the means ± S.E.M. obtained from 5 microscopic fields. $^{**}P < 0.01$, $^{***}P < 0.001$ in comparison with vehicle.

3.5. Effects on peritoneal mast cells

Significant increases in degranulated mast cells were observed after incubation of mesenteric tissue with 1,8-cineole or compound 48/80 (Fig. 5). The percentage of degranulated cells in vehicle controls was 13 ± 3%. Responses to the mast cell degranulating agent, compound 48/80, were qualitatively similar to those of 1,8-cineole. At the concentrations of 0.1, 0.3 and 1.0 μl/ml, 1,8-cineole induced degranulation of mast cells in the order of 61 ± 4, 94 ± 2 and 87 ± 6%, respectively. Compound 48/80 induced 87 ± 7, 96 ± 1 and 97 ± 2% degranulation respectively, at the concentrations of 0.03, 0.1 and 0.3 μg/ml in the incubation medium.

4. Discussion

The present results show that subplantar injection of 1,8-cineole, a terpenoid oxide found in many essential oils, produces long-lasting dose-dependent oedema in the hind-paw of rats. To understand the mechanisms involved in 1,8-cineole-induced paw oedema, the effect of several pharmacological agents on this reaction was analyzed. Among the drugs tested, the antihistaminic diphenhydramine, the antiserotonergic methysergide, and a potent antagonist of both histamine and serotonin, cyproheptadine (Maling et al., 1974), significantly suppressed the paw oedema. Since mast cells, following their activation are known to release several preformed and soluble mediators, including histamine and serotonin (Schwartz and Austen, 1984), which are considered largely responsible for hind-paw oedema in the rat (Rowley and Benditt, 1956), we verified the possibility of mast cell activation in 1,8-cineole-induced paw oedema.

Repeated treatment with compound 48/80 to achieve depletion of mast cell-derived mediators has been a successful strategy in defining the role of mast cells in a variety of experimental situations (Di Rosa et al., 1971). The present study showed an almost complete absence of oedema in response to 1,8-cineole in 48/80-pretreated animals. The data obtained for ketotifen, a mast cell stabilizer that blocks the release of chemical mediators (Greenwood, 1982), show that it also caused effective blockade of 1,8-cineole-induced paw oedema. In addition, 1,8-cineole was able to produce degranulation of peritoneal mast cells in vitro in a manner similar to 48/80. These observations indicate that mast cells play a predominant role in the paw oedema induced by 1,8-cineole.

Paw oedema provoked by subplantar injection of 1,8-cineole appeared to be more sustained and longer lasting than that produced by compound 48/80. However, sys-

temic treatment with compound 48/80 almost completely prevented the oedema response to 1,8-cineole. Presumably two different mechanisms are involved in the oedema-inducing effect of 1,8-cineole. Firstly, 1,8-cineole may promote mast cell degranulation in a manner similar to compound 48/80 and secondly, it may stimulate de novo synthesis of other mediators or factors that could give rise to a more sustained oedema reaction. It has been proposed that following mast cell activation, mast cells not only liberate preformed and stored mediators like histamine and serotonin but also synthesize a number of mediators that include arachidonic metabolites such as prostanoids and leukotrienes as well as platelet activating factor and adenosine (Befus et al., 1988).

The present data also reveal that the cyclooxygenase inhibitor, indomethacin, and the corticosteroid, dexamethasone, were ineffective in inhibiting in the first hour the oedema response to 1,8-cineole, which largely involves the amines, histamine and serotonin. This observation is consistent with earlier reports that show the ineffectiveness of cortisone and phenylbutazone in modifying dextran-induced oedema in which histamine and serotonin play a predominant role (Stucki and Thompson, 1958). Both indomethacin and dexamethasone, however, were found to be effective in inhibiting at 2 h the peak oedema as well as the subsequent oedema caused by 1,8-cineole, which reinforces our contention that 1,8-cineole not only liberates histamine and serotonin but also may promote the synthesis of some other mediators.

The mechanism(s) by which 1,8-cineole activates mast cells and liberates chemical mediators is not clear from this study. A number of substances such as substance P (Foreman et al., 1983), phospholipases and lipoxygenases (Kennerly et al., 1979; Wang and Teng, 1990) and adenosine (Fozard et al., 1996) can cause mast cell activation and release of mediators. In the present experiments, neither nordihydroguiretic acid, a lipoxygenase inhibitor, nor systemic depletion of substance P by capsaicin treatment caused any significant change in the paw oedema response to 1,8-cineole. Interestingly rats pretreated with thalidomide, a selective inhibitor of tumor necrosis factor (Sampaio et al., 1994), showed significantly less oedema following subplantar injection of 1,8-cineole. Whether this means that mast cell activation produces tumor necrosis factor-α (TNF-α) remains to be determined. Moreover, thalidomide is being considered as a clinical drug of choice to suppress the inflammatory attack associated with leprosy (Sampaio et al., 1993). Therefore it is possible that TNF-α may have some influence on mast cell degranulation.

In conclusion, the present data provide direct evidence of the ability of 1,8-cineole to induce hind-paw oedema in the rat and indicate a key role for mast cells in its manifestation. Mast cells are preferentially located in the skin epithelium, in close proximity to blood and lymphatic vessels, and the release of mediators from these cells has been implicated in the pathogenesis of many skin disorders including urticaria and psoriasis, hypersensitivity and other inflammatory reactions, cell toxicity, vascular growth and integrity and in fibrosis (Benyon et al., 1987; Befus et al., 1988). Cineole has been considered a skin irritant on the basis of anecdotal evidence (Lassak and McCarthy, 1983). Further, contact dermatitis and adverse skin reactions following ingestion of the tea tree (*Melaleuca alternifolia*) oil has been attributed to its major component, 1,8-cineole (De Groot and Weyland, 1992; Elliott, 1993). In the light of these observations and the present finding of its ability to stimulate the release of mast cell mediators, caution must be exercised in the use of 1,8-cineole for aromatherapy.

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