Ethnopharmacological communication

α-Pinene, linalool, and 1-octanol contribute to the topical anti-inflammatory and analgesic activities of frankincense by inhibiting COX-2

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1. Introduction

According to traditional Chinese medicine, frankincense from Boswellia carterii (Chinese: Rò Xiang) could relieve pain by promoting qi circulation and activating blood stasis, and it has been widely used to treat inflammation and pain, including muscle pain, dysmenorrhea, epigastric pain, rheumatic pain (Batista et al., 2010). Traditionally, frankincense was soaked in water or sesame oil and applied externally to the skin for pain relief. As a topical analgesic, frankincense provides pain relief and targets pain precisely without the side effects of oral administration (Pan et al., 2015).

Frankincense contains 60–85% resins (mixture of terpenes), 6–30% gums (mixture of polysaccharides), and 5–15% essential oils (Batista et al., 2010; Pan et al., 2015). Boswellic acids in the resinous part are inhibitors of 5-lipoxygenase and thought to be responsible for anti-inflammatory activity (Verhoff et al., 2014); the gum portion may contribute to anti-oxidative effects (Batista et al., 2010); and the essential oil, a mixture of terpenes and sesquiterpenes or their derivatives, has anti-tumor (Singh et al., 2008) and antibacterial activities (Batista et al., 2010). More than three hundred volatiles have been identified in the essential oil, common compounds were α-pinene, 1-octanol, linalool, octyl acetate, α-thujene, and (E)-β-ocimene et al. (Pan et al., 2015). Although many terpenes, including α-pinene and linalool have anti-inflammatory and/or analgesic activities, however, to our knowledge there are no reports on the contribution of the essential oil portion to the topical anti-inflammatory and analgesic activities of frankincense. In the current study, we compared the percutaneous absorption efficiencies of frankincense oil extract (FOE) and water extract (FWE) by identifying the anti-inflammatory and analgesic effects on in vivo animal models. The potential effective components were also elucidated.

2. Materials and methods

2.1. Preparation of plant extracts

Frankincense from Boswellia carterii (150 g), originating from the Ethiopia, was soaked in 1 L sesame oil or water for 7 days at

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room temperature and subsequently incubated at 100 °C for 2 h. The oil and water extracts were filtered and collected.

2.2. GC–MS analysis

To test the chemical components, extracts of frankincense were detected by GC–MS method as described in Supplementary materials. Three components (α-pinene, 0.072 mg/mL; linalool, 0.0593 mg/mL; and 1-octanol, 0.359 mg/mL) were identified in the FOE (Fig. 1a). The total amounts of the three components extracted by oil were much higher than those extracted by water (Supplementary materials). Thus, α-pinene, linalool, and 1-octanol were determined.
chosen as possible active ingredients for further evaluation.

2.3. Animals

The care and use of animals and experimental protocols for this study were performed according to the Guide for Animal Experimentation, South-Central University for Nationalities and the Committee of Research Facilities for Laboratory Animal Sciences, South-Central University for Nationalities, China (Permit Number: 2013-SCUCE-AEC-007).

2.4. Anti-inflammatory and antinociceptive analysis

2.4.1. Xylene-induced ear edema

To evaluate the anti-inflammatory effect of frankincense extracts and their potential active components, a xylene-induced ear edema mouse model was induced with topical application of 20 μL xylene solvent described previously (Hosseinzadeh and Younesi, 2002). Shortly thereafter, 55 male Kunming mice (20–25 g) were randomly divided into 11 groups of five each, and each mouse was externally treated with a test substance at 0.15 mL/cm² on surface of the right ear as follows: Groups 1–8 were treated with FOE, 20% FWE, 20% FWE, α-pinene (0.072 mg/mL), linalool (0.0593 mg/mL), 1-octanol (0.359 mg/mL), and a mixture of α-pinene, linalool, and 1-octanol, respectively; Groups 9 and 10 were treated with sesame oil and water, respectively; Group 11 was treated with diclofenac diethylamine emulgel (DDE) as a positive control. Another five male mice without xylene damage (Group 12) were treated with normal. The ear thickness was measured using a micrometer at different time points. The left ear remained untreated as a normal control. The concentrations of α-pinene, linalool, and 1-octanol used here were determined from their respective concentrations in FOE.

2.4.2. Formalin-induced hind paw edema

To evaluate the anti-inflammatory and analgesic effects of frankincense extracts and their possible effective components, a formalin-inflamed mouse hind paw model was established with a formalin intradermal injection (4%, 20 μL) as described previously (Hosseinzadeh and Younesi, 2002). The experiment design was the same as that for the xylene-induced ear edema mouse model. Hind paw edema and the thermal nociceptive threshold were separately measured using a micrometer and a plantar analgesia meter at different time points after the formalin injection. The left paw remained untreated as a normal control. The mice were sacrificed after the last measurement, and the hind paw skin was immediately collected for a histological examination.

2.5. Immunohistochemistry

Four-micrometer sections were stained with hematoxylin and eosin by standard methods. The expression of COX-2 was detected as described in Supplementary materials.

2.6. Statistical analysis

All data are presented as the mean ± SEM. Statistical analysis were performed by one- or two-way ANOVA as indicated in the text using Instat software (GraphPad Software Inc., La Jolla, CA, USA). A P value less than 0.05 was considered statistically significant.

3. Results and discussion

Compared to model group, 20% FOE significantly inhibited xylene-induced ear edema development at 0.15 h (139 ± 9%, P < 0.05), whereas FOE did not (Fig. 1b). FOE exerted bidirectional regulation in a dose-dependent manner, which usually means involvement of complex active ingredients and/or multiple pathways. α-Pinene, linalool, and 1-octanol also inhibited ear edema at 0.15 h (120–135% vs 175%, P < 0.01), as did their mixture (125 ± 10%, P < 0.001; Fig. 1d). However, neither 20% nor 100% FWE exhibited a potent inhibition of ear swelling (Fig. 1c).

Compared to model group, topical administration of 20% FOE, FWE or FWE significantly raised the pain threshold of hind paw in formalin-inflamed mice at 8 and 12 h (Fig. 1h and i). There was a small but insignificant increase in the pain threshold with 20% FWE. Further, topical administration with FOE significantly reduced hind paw edema at 8 h (120 ± 7% vs 138 ± 8%, P < 0.05) and, 12 h (110 ± 3% vs 134 ± 8%, P < 0.01), whereas FWE did not. And the reduction with 20% FOE was not significant (Fig. 1e and f). α-Pinene, linalool, and 1-octanol alone and their mixture also inhibited hind paw edema at 12 h (146 ± 6%, 136 ± 3%, 155 ± 7%, and 134 ± 5%, respectively) and enhanced the pain threshold (Model, 11.0 ± 0.5 s) at 12 h (13.8 ± 0.8 s, P < 0.05; 13.8 ± 1.2 s, P = 0.05; 12.5 ± 0.7 s, P > 0.05; and 14.6 ± 0.6 s, P < 0.001, respectively; Fig. 1g and j).

These results indicate that FOE exerts greater anti-inflammatory and analgesic effects than FWE, likely due to the low water extraction efficiency of liposoluble ingredients such as α-pinene, linalool, and 1-octanol, which usually have high percutaneous absorption efficacy.

Topical application of 1.25 mg/paw boswellic acids from frankincense couldn’t show significant anti-inflammation effect (Singh et al., 2008). And that dose are thousands of times higher than the doses of α-pinene, linalool, and 1-octanol in the current study. Boswellic acids are inhibitors of 5-lipoxigenase, but had little effect on COX-2 (Verhoff et al., 2014). As an important role in the inflammatory responses of inflammatory cells and tissues, COX-2 inhibition exerts potent anti-inflammatory and analgesic effects. In the present study, compared with the control (Group 12), there was a significant increase (202 ± 20%, P = 0.001) in the expression of COX-2 in the model (Group 9; Fig. 2). After topical administration of α-pinene, linalool, 1-octanol or their mixture to the mice, the COX-2 expression decreased (115 ± 4%, P < 0.01; 116 ± 4%, P < 0.01; 129 ± 20%, P < 0.01; 111 ± 12%, P < 0.01, respectively; Fig. 2). Formalin-induced inflammatory infiltrates also significantly subsided (Fig. S1). α-Pinene and linalool are anti-inflammatory compounds prevalent in essential oils of various plant species. An intraperitoneal injection of either α-pinene or linalool in mice led to COX-2 inhibition and pain relief (Batista et al., 2010; Kim et al., 2015). However, to our knowledge, there is no report about their topical anti-inflammatory and analgesic activities. As for 1-octanol, a common compound, there is little information available concerning its pharmacological properties. Thus, these results in current study for the first time indicated that α-pinene, linalool, and 1-octanol contributed to the topical anti-inflammatory and analgesic activities of frankincense by inhibiting COX-2 protein expression in the inflammatory tissues. As the positive control in the current study, DDE showed potential anti-inflammatory and analgesic effects. Concerning the analgesic effect, FOE and the three compounds were more potent than DDE. Concerning the anti-inflammation effect, DDE and the three compounds showed similar effects which were more potent than FOE. Moreover, multiple pathways, including MAPKs, NF-kB, etc., involve in the anti-inflammatory effect of essential oils (Kim et al., 2015). Therefore, further study is needed to clarify the involved pathways.

4. Conclusion

These results indicate that topical administration of FOE, which includes α-pinene, linalool, and 1-octanol as active ingredients,
exhibit significantly anti-inflammatory and analgesic effects through inhibiting nociceptive stimulus-induced inflammatory infiltrates and COX-2 overexpression. FOE may serve as a promising potent therapeutic agent for treatment of chronic pain.

Competing financial interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2015.12.039.

References


