RURAL INDUSTRIES RESEARCH
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Anti-inflammatory Activity of Tea Tree Oil

A report for the Rural Industries Research and Development Corporation

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Executive Summary

Tea tree oil (TTO) is the essential oil steam distilled from the Australian native plant, *Melaleuca alternifolia*. It is a complex mixture of approximately 100 terpenes and hydrocarbons, the main component being terpinen-4-ol which comprises at least 30% of the oil. Besides anecdotal evidence for the anti-inflammatory properties of TTO, components of the oil have been demonstrated to show anti-inflammatory activity in experimental inflammation in animals. For example, in a carageenan-induced hind paw oedema model in rats, terpinen-4-ol had anti-inflammatory activity when applied topically in mg amounts [1]. In the same model, α-terpineol (a minor component of TTO comprising approximately 3% of the oil) was anti-inflammatory when administered subcutaneously as a 7.5% mixture with linalool [2]. However, the mechanisms of the anti-inflammatory effects of TTO remain undefined.

In our first study of the anti-inflammatory activity of TTO *in vitro*, human peripheral blood monocytes were used as a model for tissue macrophages. Upon activation with molecules such as lipopolysaccharide (LPS), these cells produce many mediators including the central mediators of inflammation, tumour necrosis factor-α (TNFα) and interleukin-1β (IL-1β). Other important monocyte/macrophage-derived mediators of inflammation include IL-8, IL-10 and prostaglandin E_2 (PGE_2). Together with other products of activated macrophages, these molecules can damage tissue or, in turn, activate other cells to produce pro-inflammatory mediators. It was hypothesised that if anti-inflammatory, TTO would reduce the production *in vitro* of TNFα, IL-1β, IL-8, and PGE_2 by LPS-activated monocytes.

TTO emulsified by sonication in a glass tube into culture medium containing 10% fetal calf serum (FCS) was toxic for monocytes at a concentration of 0.016% v/v. However, the water soluble components of TTO at concentrations equivalent to 0.125% significantly suppressed LPS-induced production of TNFα, IL-1β and IL-10 (by approximately 50%) and PGE_2 (by approximately 30%) after 40 h. Gas chromatography/ mass spectrometry identified terpinen-4-ol (42%), α-terpineol (3%) and 1,8-cineole (2%, respectively, of TTO) as the water soluble components of TTO. When these components were examined individually, only terpinen-4-ol suppressed the production after 40 h of TNFα, IL-1β, IL-8, IL-10 and PGE_2 by LPS-activated monocytes. We concluded that the water-soluble components of TTO can suppress pro-inflammatory mediator production by activated human monocytes.

Oxygen derived reactive species (ODRS) such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radical, as well as hypochlorous acid and various chloramines [3], are formed by activated macrophages and neutrophils. ODRS play an important role in immunological host defence, providing anti-microbial, anti-viral and anti-tumour activity, as well as being involved in apoptosis and cell survival [3, 4,]. However, increased levels of ODRS (such as those generated during chronic and acute inflammatory diseases) are cytotoxic and may cause tissue damage through lipid peroxidation, oxidation of amino acid side chains, protein cross-linking and fragmentation, and DNA damage [5-7].

In our second study of the anti-inflammatory properties of TTO, we examined the effects of TTO on the production of ODRS (superoxide) in monocytes and neutrophils stimulated *in vitro*. In the absence of toxicity, the water-soluble fraction of TTO had no significant effect on agonist-stimulated superoxide production by neutrophils, but significantly and dose-dependently suppressed agonist-stimulated superoxide production by monocytes. When the water-soluble components were examined individually, terpinen-4-ol significantly suppressed N-formyl-methionyl-leucyl-phenylalanine (fMLP)- and LPS- but not phorbol myristate

acetate (PMA)-stimulated superoxide production; α-terpineol significantly suppressed fMLP-, LPS- and PMA-stimulated superoxide production; 1,8-cineole was without effect. From this study we concluded that TTO components suppress the production of superoxide by monocytes, but not neutrophils, suggesting the potential for selective regulation of cell types by these components during inflammation.

The physiological relevance of these studies is high as it implies TTO has potential as an anti-inflammatory agent. The results suggest TTO contains water-soluble components, specifically terpinen-4-ol and α-terpineol, that may selectively regulate cell function during inflammation, in particular monocyte activity, and following topical application may control inflammatory responses to foreign antigens in the skin. TTO may enable neutrophils to be fully active in an acute inflammatory response and eliminate foreign antigens, while suppressing monocyte inflammatory mediator and superoxide production and thereby preventing oxidative tissue damage that may be seen in more chronic inflammatory states.

The potential of TTO as a topical anti-inflammatory agent requires confirmation through documentation of a reduction of inflammatory cells and mediators in skin after application of TTO.

Chapter 4: References

1 Pongprayoon U, Soontornsaratune P, Jarikasem S, Sematong T, Wasuwat S, Claeson P. Topical anti-inflammatory activity of the major lipophilic constituents of the rhizome *Zingiber cassumunar*. Part I: The essential oil. Phytomedicine 1996/97;3:319-22.

2 Moretti MDL, Peana AT, Satta M. A study on anti-inflammatory and peripheral analgesic action of *Salvia sclarea* oil and its main components. J Essent Oil Res 1997;9:199-204.

3 Bogdan C, Röllinghoff M, Diefenbach A. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr Opin Im 2000;12:64-76.

4 Lander HM. An essential role for free radicals and derived species in signal transduction. FASEB J 1997;11:118-24.

5 Davies KJ. Protein damage and degradation by oxygen radicals. I. General aspects. J Biol Chem 1987;262:9895-901.

6 Davies KJ, Goldberg AL. Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. J Biol Chem 1987;262:8220-6.

7 Li N, Karin M. Is NF-κB the sensor of oxidative stress? FASEB J 1999;13:1137-43.

8 Walsh LJ, Longstaff J. The antimicrobial effects of an essential oil on selected oral pathogens. Periodontology 1987;8:11-5.

9 Altman PM. Australian tea tree oil. Aust J Pharm 1988;69:276-8.

10 Altman PM. Australian tea tree oil - a natural antiseptic. Aust J Biotechnol 1989;3:247-8.

11 Carson CF, Riley TV. The antimicrobial activity of tea tree oil. Med J Aust 1994a;160:236.

12 Carson CF, Riley TV. Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia*. Lett Appl Microbiol 1994b;19:24-5.

13 Carson CF, Riley TV. Antimicrobial susceptibility of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol 1995a;78:264-9.

14 Carson CF, Riley TV. Toxicity of the essential oil of *Melaleuca alternifolia* or tea tree oil. J Toxicol Clin Toxicol 1995b;33:193-4.

15 Carson CF, Cookson BD, Farrelly HD, Riley TV. Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia*. J Antimicrob Chemother 1995c;35:421-4.

16 Hammer KA, Carson CF, Riley TV. Susceptibility of transient and commensal skin flora to the essential oil of *Melaleuca alternifolia*. Am J Infect Control 1996;24:186-9.

17 Ocete MA, Risco S, Zarzuelo A, Jimenez J. Pharmacological activity of the essential oil of *Bupleurum gibraltaricum*: anti-inflammatory activity and effects on isolated rat uteri. J Ethnopharmacol 1989;25:305-13.

18 Martin S, Padilla E, Ocete MA, Galvez J, Jimenez J, Zarzuelo A. Anti-inflammatory activity of the essential oil of *Bupleurum fruticescens.* Planta Med 1993;59:533-6.

19 Essential oils - oil of *Melaleuca*, terpinen-4-ol (tea tree oil). ISO-4730 (1996) International Organisation for Standardisation, Geneva, Switzerland.

20 Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli D, Hamilton JA. Potential antiinflammatory effects of interleukin 4: suppression of human monocyte TNFα, interleukin 1 and prostaglandin E_2. Proc Natl Acad Sci USA 1989;86:3803-7.

21 Hart PH, Ahern MJ, Jones CA, Jones KL, Smith MD, Finlay-Jones JJ. Synovial fluid macrophages and blood monocytes differ in their responses to interleukin-4. J Immunol 1993;151:3370-80.

22 Soderberg TA, Johansson A, Gref R. Toxic effects of some conifer resin acids and tea tree oil on human epithelial and fibroblast cells. Toxicology 1996;107:99-109.
23 Hayes AJ, Leach DN, Markham JL. In vitro cytotoxicity of Australian tea tree oil using human cell lines. J Essent Oil Res 1997;9:575-82.
24 Obata Y, Takayama K, Machida Y, Nagai T. Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des Delivery 1991;8:137-44.
25 Okabe H, Obata Y, Takayama K, Nagai T. Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug Des Delivery 1990;6:229-38.
26 Magnusson BM, Runn P, Koskinen LOD. Terpene-enhanced transdermal permeation of water and ethanol in human epidermis. Acta Dermato-Venereol 1997;77:264-7.
27 Juergens UR, Stober M, Vetter H. Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1,8-cineole) in human monocytes in vitro. Eur J Med Res 1998a;3:508-10.
28 Juergens UR, Stober M, Schmidt-Schilling L, Kleuyer T, Vetter H. Antiinflammatory effects of eucalyptol (1,8-cineole) in bronchial asthma: Inhibition of arachidonic acid metabolism in human blood monocytes ex vivo. Eur J Med Res 1998b;3:407-12.
29 Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol 1992;10:411-36.
30 Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. Arth Rheum 1993;12:1681-90.
31 Williams LR. Clonal production of tea tree oil high in terpinen-4-ol for use in formulation for the treatment of thrush. Complement Ther Nurs Midwif 1998;4:133-6.
32 Ferrante A, Thong YH. A rapid one-step procedure for purification of mononuclear and polymorphonuclear leukocytes from human blood using a modification of the Hypaque-Ficoll technique. J Immunol Methods 1978;24:389-93.
33 Gyllenhammar H. Lucigenin chemiluminescence in the assessment of neutrophil superoxide production. J Immunol Methods 1986;97:209-13.
34 Pippin MA, Pabst KM, Pabst MJ, Haney L, Dabbous MKH. Superoxide release by neutrophils is inhibited by tea tree oil. J Dent Res 1994;73:259.
35 Santos FA, Rao VSN. Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constitutent of eucalyptus and rosemary oils. Eur J Pharm 1997;331:253-8