Anti-inflammatory Activity of Tea Tree Oil

A report for the Rural Industries Research and Development Corporation

by Professor John Finlay-Jones, Dr Prue Hart, Associate Professor Thomas Riley, and Ms Christine Carson

February 2001

RIRDC Publication No 01/10
RIRDC Project No UF-5A
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 E2 (PGE2). 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 PGE2 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 PGE2 (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 PGE2 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


35 Santos FA, Rao VSN. Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constituent of eucalyptus and rosemary oils. Eur J Pharm 1997;331:253-8