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An Investigation of the Actions of the Essential oils of Manuka (*Leptospermum scoparium*) and Kanuka (*Kunzea ericoides*), Myrtaceae on Guinea-pig Smooth Muscle

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Abstract

The two New Zealand tea-tree oils, Manuka (*Leptospermum scoparium* J.R. et G. Forst) and Kanuka (*Kunzea ericoides* (A. Rich) J. Thompson), Myrtaceae have been used as folk medicines for treating diarrhoea, colds and inflammation but their pharmacological action has not been investigated. Their mode of action was therefore studied on the field-stimulated guinea-pig ileum.

Both Manuka and Kanuka oils induced a spasmolytic effect but Kanuka produced an initial contraction. The spasmolytic action of both oils was the result of a post-synaptic mechanism. Action involving adrenoceptors or cGMP was not considered likely, neither did the oils behave like calcium- or potassium-channel openers. There is some evidence that Manuka acts through cAMP whereas the mode of action of Kanuka is as yet undetermined.

The results indicate that the use of these oils as relaxants in aromatherapy might be valid, although their mode of action is not identical.

Manuka (*Leptospermum scoparium* J. R. et G. Forst) and Kanuka (*Kunzea ericoides* (A. Rich) J. Thompson formerly *Leptospermum ericoides*), Myrtaceae, are both indigenous to New Zealand where they are known as tea-tree because, it is said, Captain Cook used both types of leaf as tea equivalents (Brooker et al 1987). There is, however, no resemblance between real tea, *Camellia sinensis* and the taste or odour of these New Zealand species. The tea-tree from Australia (*Melaleuca alternifolia*, Myrtaceae) is also completely different having an essential oil of a different composition which is used successfully as an antimicrobial and antifungal agent in creams, soaps, shampoos and other preparations.

The folk-medicinal uses of New Zealand and Australian tea-tree are similar and there is interest in possible commercial application of the New Zealand species. Brooker et al (1987) report a variety of uses for extracts of Manuka and Kanuka including leaves for vapour baths to stop coughing and cold symptoms, boiled seed capsules to give a decoction for external use in inflammation or internal for diarrhoea, and water from boiled bark for inflammation. It has been shown that honeys derived from Kanuka and Manuka blossom have antibacterial activity against *Staphylococcus aureus* (Allen et al 1991) and more recently it has been reported that Manuka honey is active against *Helicobacter pylori* (Somai et al 1994). Manuka contains leptospermone which has antihelmintic properties and is closely related to compounds with similar properties found in ferns (Brooker et al 1987).

Because of the folk-medicinal use of Manuka and Kanuka and their use in aromatherapy, the bioactivity of their essential oils has been studied against microorganisms and on the smooth muscle of guinea-pig ileum; their anti-oxidant activity has also been studied (Lis-Balchin et al 1996). This paper describes an investigation of the mechanisms by which Manuka and Kanuka essential oils produce their spasmolytic action.

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Materials and Methods

Materials

Essential oils were obtained from Absolute Essential, Auckland, New Zealand, and were prepared by

steam distillation of leaves and branchlets (Kanuka) or bushes (Manuka). Previous studies (Lis-Balchin et al 1996) had shown considerable variation in the bioactivity of Manuka samples from different areas of New Zealand; the sample used in this study was one with high antimicrobial activity. Essential oils were diluted in methanol. Drugs were obtained from Sigma (Poole, Dorset, UK) and were dissolved in isotonic saline. CDP 840 (*R*-(+)-4-[2-(3-cyclopentoxy-4-methoxyphenyl)-2-phenyl) was a gift from Dr B. Hughes of Celltech Therapeutics. Concentrations of drugs are expressed in molarities but the concentrations of essential oils are in g L^{-1}.

Pharmacological studies

The activity of the oils was investigated on the guinea-pig field-stimulated isolated ileum which was mounted in a 25-mL organ bath containing Krebs solution at 34°C and gassed with 95% oxygen in carbon dioxide. Field stimulation was applied by means of two parallel platinum electrodes placed either side of the tissue and attached to a stimulator (0·1 Hz, 0·5-ms pulse, 70 V). Changes in tension were recorded with an isometric transducer attached to a pen recorder. All experiments were repeated at least four times on different strips of ileum; where appropriate results are expressed as mean percentage inhibition ± s.e.

Essential oils diluted in methanol (≤ 0·2 mL) were added to the organ bath. The maximum volume of methanol used had no effect on the experiments.

Results

Field stimulation of guinea-pig ileum induced regular and reproducible contractions which were unaffected by the administration of 0·2 mL methanol. Manuka oil reduced the contractions dose-dependently with an IC50 (the concentration having half the maximum effect) of 1×10^{-5} (Figure 1). The reduced response was maintained for as long as the oil was in contact with the tissue and recovery occurred within 3 min after a single wash.

In the presence of a low concentration (6×10^{-6}) of Kanuka oil the size of the field-stimulated contraction of guinea-pig ileum was increased for about 2 min before development of a small amount of inhibition. With higher concentrations there was an initial increase in tone followed by a rapid reduction in the size of the contraction. The IC50 for this spasmolytic effect was 5×10^{-5} (Figure 1) and recovery occurred within 12 min after a single wash. At the IC50 concentration, the electrically-induced contraction was initially increased by 25%.

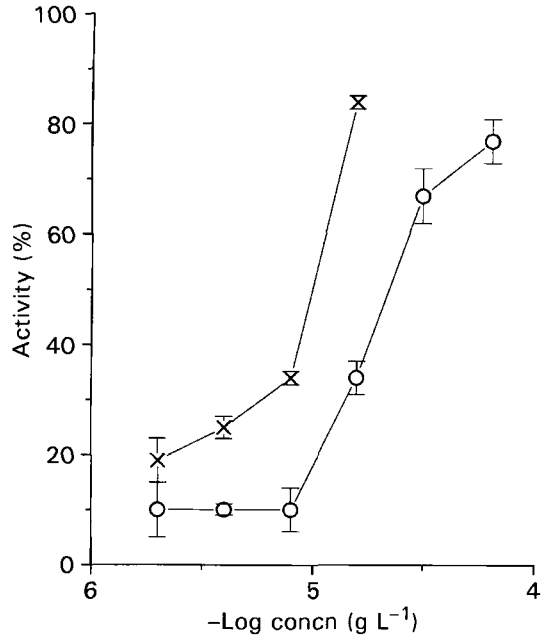

Figure 1. Dose–response curve for the spasmolytic activity of Kanuka (○) and Manuka (×) essential oils on field-stimulated guinea-pig ileum.

Contractions induced by acetylcholine (10^{-7}) and histamine (10^{-7}) were inhibited by 15·4 ± 1·0 and 19·2 ± 1·2%, respectively, by a concentration of Manuka oil which produced a significant inhibition of the contraction induced by field stimulation. Similarly, a concentration of Kanuka oil (4×10^{-6}) which reduced the contraction caused by electrical stimulation by 34·7 ± 3·2% resulted in 63·6 ± 3·1% inhibition of the response to exogenous acetylcholine and 59·6 ± 12·3% inhibition of the response to histamine.

Noradrenaline (8×10^{-7}), reduced the size of the field-stimulated contraction by 68·8 ± 1·5% and this spasmolytic action was reduced to 17·8 ± 2·4% inhibition in the presence of phentolamine (10^{-6}) plus propranolol (10^{-6}). These concentrations of adrenoceptor antagonists had no effect on the spasmolytic effect of Manuka oil (38·1 ± 1·4% alone and 37·6 ± 2·5% with antagonists). Similarly, a concentration of phentolamine and propranolol which reduced the inhibitory response of noradrenaline from 47·6 ± 2·2 to 18·5 ± 1·0% had no significant effect on the spasmolytic action of Kanuka oil (59·7 ± 4·6% alone and 53·7 ± 3·3% with antagonists).

A concentration of trequinsin (10^{-7}) which potentiated the spasmolytic action of isoprenaline (22·6 ± 2·2 to 41·8 ± 3·5%) had less effect on the response to Manuka oil (15·0 ± 2·2 to 25·3 ± 4·6%, $P = 0.019$) and no effect on Kanuka oil (27·3 ± 3·4 to 28·2 ± 4·2%). The selective phosphodiesterase inhibitor CDP 840 (10^{-7}) potentiated the response

to isoprenaline (23·6 ± 2·5 to 36·6 ± 3·3%) but had no effect on the spasmolytic activity of Manuka (37·0 ± 7·6 to 37·6 ± 8·8%) or Kanuka oil (27·1 ± 4·9 to 32·1 ± 5·1%).

ODQ, at a concentration (10^{-6}) which reversed the inhibition of the twitch response produced by sodium nitroprusside, had no effect on the spasmolytic action of Manuka (37·5 ± 2·6 to 39·8 ± 3·1) or Kanuka (33·5 ± 2·5 to 34·1 ± 2·6) oils. The K^+-contracted ileum was relaxed by Manuka (33·3 ± 3·5%) and Kanuka (39·9 ± 3·8%) at a concentration which produced a spasmolytic effect on the field-stimulated preparation, a response not seen with cromakalin (10^{-6}). When calcium-free Krebs was replaced by normal Krebs, the response to field-stimulation recovered in 5·5 ± 1·5 min. In the presence of nifedipine (10^{-6}), recovery was delayed to 29·8 ± 5·5 min, but no such delay occurred with Manuka (4·2 ± 1·7 min) or Kanuka (3·5 ± 1·9 min).

Discussion

The field-stimulated guinea-pig ileum preparation is appropriate for the examination of spasmolytic activity because it enables immediate distinction between neurogenic and myogenic action and subsequent examination of the site of action. Both Manuka and Kanuka oil acted post-synaptically, as was shown by their ability to inhibit contractions induced by acetylcholine and histamine. An atropine-like effect was ruled out because neither compound elicited any selective antagonism of acetylcholine compared with histamine. The inability of inhibitory concentrations of adrenoceptor antagonists to affect the spasmolytic activity of the oils demonstrated that they were probably not acting through adrenoceptors.

Relaxation produced by sympathomimetics involves an increase in levels of cAMP whilst nitric oxide donors act through an increase in cGMP. It was thus possible that Manuka and Kanuka oil were raising the levels of these second messengers. The positive response seen with Manuka in the presence of trequinsin implies action through adenylate cyclase but this did not apply to Kanuka and neither oil seemed to act through guanylate cyclase. It is also possible that the spasmolytic activity involves more than one site of action but neither oil acted in a similar manner to a calcium-channel antagonist or a potassium-channel opener.

In conclusion, this study has demonstrated that the spasmolytic activity of Manuka oil and Kanuka oil is similar to some extent, but it must be remembered that Kanuka oil produced an initial increase in tone of smooth muscle. This spasmogenic activity might be a result of the high monoterpene content of Kanuka oil, which is particularly rich in α-pinene (Lis-Balchin et al 1996). The two oils were also bioactive against microorganisms (Lis-Balchin et al 1996).

This study has also demonstrated that the spasmolytic action of Manuka oil and Kanuka oil is myogenic and that for Manuka, cAMP might be involved. The use of the two oils as relaxant oils in aromatherapy might be justified to some extent although differences in the mode of action of the two oils is very apparent and Kanuka has a strong initial spasmogenic action which could interfere with the overall relaxant effect. The difference in activity is not unexpected in view of the differences in their chemical composition—Manuka oil contains a high percentage of sesquiterpenes whereas Kanuka contains monoterpenes (Lis-Balchin et al 1996).

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