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Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes

P. H. Hart[1], C. Brand[1], C. F. Carson[2], T. V. Riley[2], R. H. Prager[3] and J. J. Finlay-Jones[1]

[1] Department of Microbiology and Infectious Diseases, School of Medicine, Flinders University, GPO Box 2100, Adelaide, 5001, Australia, Fax: 61 8 8276 8658, e-mail: Prue.Hart@flinders.edu.au

[2] Department of Microbiology, University of Western Australia, Nedlands, Australia 6907

[3] School of Chemistry, Physics and Earth Sciences, Flinders University, GPO Box 2100, Adelaide, 5001, Australia

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Abstract. *Objective and Design:* To evaluate potential anti-inflammatory properties of tea tree oil, the essential oil steam distilled from the Australian native plant, *Melaleuca alternifolia*.
Material and Methods: The ability of tea tree oil to reduce the production in vitro of tumour necrosis factor-α (TNFα), interleukin (IL)-1β, IL-8, IL-10 and prostaglandin E_2 (PGE_2) by lipopolysaccharide (LPS)-activated human peripheral blood monocytes was examined.
Results: Tea tree oil 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 tea tree oil 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 tea tree oil) as the water soluble components of tea tree oil. 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.
Conclusion: The water-soluble components of tea tree oil can suppress pro-inflammatory mediator production by activated human monocytes.

Key words: Tea tree oil – Monocytes – Interleukin-1 – Tumour necrosis factor-α – Prostaglandin E_2

Introduction

Tea tree oil is the essential oil steam distilled from the Australian native plant, *Melaleuca alternifolia.* Tea tree oil contains over 100 components, the majority being monoterpene and sesquiterpene hydrocarbons and their alcohols. The antibacterial properties of tea tree oil have now been well documented, and there are susceptibility data on a wide range of bacteria [1–9]. There is also considerable information identifying the components of tea tree oil active against bacteria and yeasts [6]. Until recently there have only been anecdotal claims about tea tree oil's anti-inflammatory activity. The only specific report on anti-inflammatory properties of tea tree oil has been of an in vitro study published as an abstract [10] in which the addition of tea tree oil to lipopolysaccharide-primed neutrophils inhibited superoxide release by approximately 85%. The concentration of tea tree oil used was 0.05% v/v.

Several studies have investigated the anti-inflammatory properties of compounds also found in tea tree oil in a rat carrageenan-induced hind paw oedema model [11–14]. These studies investigated the immunomodulatory effects of oils from *Bupleurum gibraltaricum*, *Bupleurum fruticescens*, *Zingiber cassumunar* and *Salvia sclarea*, respectively. Collectively, these studies concluded that α-pinene [11, 12], α-terpinene [13], terpinen-4-ol [13], α-terpineol [14] and linalool [14] may have direct or indirect anti-inflammatory activity. The implications of these studies for the activity of tea tree oil are uncertain since the composition of the oils and the concentrations used were generally in excess of those found in tea tree oil. However, they go some way to identifying essential components of tea tree oil which possess anti-inflammatory activity. Whether there are synergistic or antagonistic interactions of several potential immunomodulators when they are in the same chemical mixture is not known. Some of the components of tea tree oil previously shown to have anti-bacterial properties, e. g. terpinen-4-ol and α-terpineol, are also anti-inflammatory in vivo [6].

In this study of the anti-inflammatory activity of tea tree oil in vitro, human peripheral blood monocytes were used as

Correspondence to: P. H. Hart

a model for tissue macrophages. Upon activation with molecules such as LPS, these cells produce many mediators including the central mediators of inflammation, TNFα and IL-1β. Other important monocyte/macrophage-derived mediators of inflammation include IL-8, IL-10 and 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, tea tree oil would reduce the production in vitro of TNFα, IL-1β, IL-8, IL-10 and PGE_2 by LPS-activated monocytes.

Materials and methods

Tea tree oil and its major components

Tea tree oil was kindly provided by Australian Plantations (Wyrallah, NSW, Australia). The levels of 15 major components of the batch of tea tree oil used in all experiments (97/03) was examined by Wollongbar Agricultural Institute, Wollongbar, Australia, according to the international standard for tea tree oil [15] and were as follows: terpinen-4-ol (41.6%), γ-terpinene (21.5%), α-terpinene (10.0%), terpinolene (3.5%), α-terpineol (3.1%), α-pinene (2.4%), 1,8-cineole (2.0%), p-cymene (1.8%), aromadendrene (1.1%), δ-cadinene (1.0%), limonene (0.9%), ledene (0.9%), globulol (0.5%), sabinene (0.4%) and viridiflorol (0.2%) (Table 1). For individual study, terpinen-4-ol and α-terpineol were obtained from Fluka, Buchs, Switzerland and 1,8-cineole from Sigma Chemical Co., St Louis, MO.

Monocyte isolation and culture

Human monocytes were isolated from peripheral blood as published [16, 17] to >93% purity by countercurrent centrifugal elutriation and cultured in RPMI-1640 medium (Cytosystems, Castle Hill, Australia) supplemented with 13.3 mM $NaHCO_3$, 2 mM glutamine, 50 mM β-mercaptoethanol, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 nM 3-(*N*-morpholino)propanesulphonic acid with an osmolality of 290 mmol/kg H_2O ('complete RPMI'). During isolation and subsequent culture of all cells, extreme care was taken to limit LPS contamination of buffers and culture fluids [16, 17].

For measurement of regulation of cytokine production, freshly isolated monocytes were cultured at 10^6 cells/ml with tea tree oil or its water-soluble components and, unless otherwise indicated, 1% fetal calf serum (FCS). LPS from *Escherichia coli* 0111:B4 (Sigma) was added to give a final concentration of 500 ng/ml. Triplicate cultures for each test variable were incubated at 37°C in 5% CO_2. After 20 or 40 h, cultures were centrifuged and the supernatants harvested for measurement of TNFα, IL-1β, IL-8, IL-10 and PGE_2. For cultures of adherent cells, polystyrene 24-well tissue culture plates (Falcon, Becton Dickinson, Lincoln Park, NJ) were used.

Measurement of toxicity of tea tree oil and its water soluble components

Metabolically active cells were enumerated using the CellTiter 96 AQ_{ueous} non-radioactive cell proliferation assay (Promega Corporation, Madison, WI). Monocytes (2×10^5/200 µl) were cultured under adherent conditions for 17 or 37 h with tea tree oil or its water-soluble components, with or without LPS. The MTS/PMS substrate (20 µl) was added and the amount of formazan product measured spectrophotometrically at 490 nm hourly for the next 4 h.

ELISAs for TNFα, IL-1β, IL-8 and IL-10

Culture supernatants were stored at –20°C until used. TNFα, IL-8 and IL-10 were measured by sandwich ELISA using mAbs to human TNFα, IL-8 and IL-10 purchased as antibody pairs from PharMingen, San Diego, CA. The antibody pair for measuring IL-1β was purchased from Endogen, Woburn, MA. One antibody from each pair was purchased as a biotinylated antibody. The assays were sensitive to levels of >40 pg/ml.

Radioimmunoassay for PGE_2

The levels of PGE_2 in the culture supernatants were determined by radioimmunoassay using competitive adsorption to dextran-coated charcoal (3H-PGE_2, Amersham, Bucks, UK; PGE_2 antiserum, Sigma). The assay was sensitive to levels of >30 pg/ml.

Solubility of tea tree oil in water

A sample of tea tree oil (500 mg) was rapidly stirred in water (50 ml) for 20 h at 20°C. The layers were separated and the aqueous layer filtered through filter paper, then extracted with ether (3 × 25 ml). The ether extracts were dried (Na_2SO_4), and the solvent carefully removed by distillation at 50°C in a closed system. The residue weighed 80 mg giving a solubility of 1.6 g/l in water. The organic phase and the oil adhering to the filter paper were extracted into ether, dried (Na_2SO_4) and evaporated as above to give 400 mg oil. Gas chromatographic analysis as above showed the composition to be 84% terpinen-4-ol, 7% α-terpineol and 3% 1,8-cineole.

For separation of tea tree oil preparations in culture medium into water and oil soluble components, very similar procedures were adopted. Immediately before separation, samples of 250 ml were vigorously shaken before separation of the aqueous and oil layers in a separating funnel. The aqueous fraction was filtered through paper, extracted with ether (2 × 25 ml), dried with sodium sulphate and the ether removed by distillation. The oily fraction, and the oil adhering to the filter paper, was extracted in a similar manner. The residues from both fractions were analysed by gas chromatography/mass spectrometry (GC/MS) (Table 1).

Expression of results

Cytokine measurements were performed on samples from triplicate cultures and the LPS-induced level was normalised to 100%. The mean values from each set of replicates were used to determine the mean + SEM for *n* donors. For comparison of responses by cell populations from a number of different donors, the Student's paired *t* test was used. A value of $P < 0.05$ was considered significant.

Results

Regulation of monocyte mediator production by tea tree oil emulsions prepared in glass tubes

To assess the regulatory properties of tea tree oil on inflammatory mediator production by monocytes, it was necessary to prepare emulsions of tea tree oil in culture medium. If tea tree oil was dissolved in propylene glycol or ethanol, the tea tree oil separated from its diluent before a dilution of 1 in 100 required for monocyte culture could be made. Instead, dilutions of tea tree oil were performed in glass tubes with medium containing 10% FCS, followed by sonication for 20 seconds immediately before use. Emulsions of tea tree oil were toxic to adherent monocytes to some extent at concentrations greater than, or equal to 0.004% (v/v). For monocytes from 4 donors, tea tree oil at 0.004% caused a mean

Table 1. Components of tea tree oil separating into aqueous and oil phases under different conditions.

Component	Concentration in tea tree oil	Glass + Sonication		Plastic		
		10% FCS		0% FCS		10% FCS
		Aqueous %	Oil %	Aqueous %	Oil %	Aqueous %
1. α-pinene	2.4	0.2	2.8	–	2.8	–
2. sabinene	0.4	–	0.9	–	0.9	–
3. α-terpinene	10.0	0.8	9.1	–	9.3	–
4. limonene	0.9	–	1.1	–	1.0	–
5. p-cymene	1.8	0.7	6.1	–	6.8	0.8
6. 1,8-cineole	2.0	2.8	1.7	3.3	1.8	2.5
7. γ-terpinene	21.5	2.3	23.0	–	21.0	1.3
8. terpinolene	3.5	–	3.8	–	3.5	–
9. terpinen-4-ol	41.6	80.4	34.3	83.8	37.0	80.8
10. α-terpineol	3.1	6.3	2.7	6.5	2.9	6.3
11. aromadendrene	1.1	–	1.4	–	1.3	–
12. ledene	0.9	–	1.2	–	1.1	–
13. δ-cadinene	1.0	–	1.2	–	1.1	–
14. globulol	0.5	–	0.5	–	0.5	–
15. viridiflorol	0.2	–	0.4	–	0.4	–

toxicity of 9 ± 5% (not significant), at 0.008% a mean toxicity of 20 ± 11% (not significant) and at 0.016%, a mean toxicity of 69 ± 17% ($P = 0.03$). No viable cells were detected after incubation of higher concentrations of tea tree oil with adherent monocytes for 20 h. As shown in Fig. 1, the suppressive properties of tea tree oil on LPS-induced TNFα production paralleled the toxic properties of tea tree oil. Similar results were detected for regulation of LPS-induced IL-1β, IL-8, IL-10 and PGE_2 (data not shown).

Removal of components of tea tree oil toxic to monocytes by dilution in plastic tubes

We next examined the effect of tea tree oil emulsions prepared in polystyrene plastic, rather than glass tubes. It was noted that the plastic adsorbed a considerable proportion of the oil; we hypothesised that this approach would allow a separation of the water soluble components into the culture medium. Initially, 10% FCS was included in the diluting medium; however, concentrations of tea tree oil greater than, or equal to 0.06% destroyed all monocytes over a 20 h incubation period. Removal of serum from the culture medium used to serially dilute the tea tree oil was then evaluated. Tea tree oil mixtures were vortexed for 1 minute immediately before further dilution. Although the diluting medium was serum-free, the monocytes were cultured in complete RPMI supplemented with 1% FCS for 20 and 40 h. Under these conditions, the monocyte toxic components in tea tree oil did not partition into the culture medium; no toxicity was detected in the cultures of monocytes harvested after 40 h (Fig. 2).

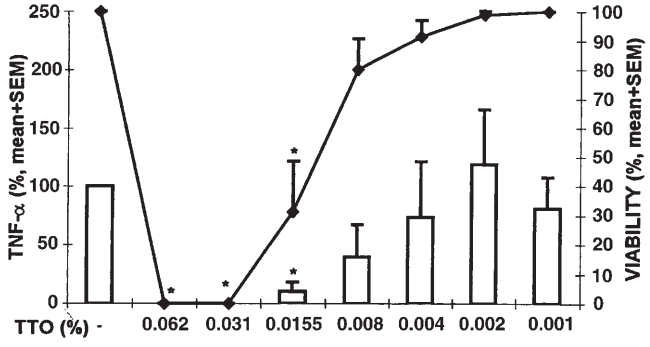

Fig. 1. The effect of tea tree oil diluted in glass tubes with medium containing 10% FCS on TNFα production by monocytes in culture for 20 h. Monocytes from 4 donors were incubated in triplicate for 20 h with LPS (500 ng/ml) and decreasing amounts of tea tree oil. The LPS-induced level was normalised to 100%; the mean result from each experiment was used to calculate the mean + SEM. Regulation of LPS-induced TNFα levels is shown by histograms (left axis). The mean percentage + SEM of viable cells in the cultures is shown by the line (right axis). An asterisk indicates a significant reduction in TNFα production or monocyte viability.

Regulation of monocyte mediator production by tea tree oil solutions prepared in plastic tubes

The tea tree oil solutions had no effect on inflammatory mediator production by monocytes incubated in the absence of LPS. Addition of LPS stimulated mediator production to levels shown in Table 2; in Fig. 2, the mediator production induced by LPS has been normalised to 100%. The dose-dependent suppressive effects of tea tree oil are shown in Fig. 2. As shown in Table 3, the suppressive properties of tea tree oil generally increased with longer incubation times.

Components of tea tree oil responsible for the anti-inflammatory activity of tea tree oil

The data presented in Figure 1 indicated that tea tree oil contained components that were toxic to monocytes in culture. When these were absent, other components of tea tree oil suppressed the production of inflammatory mediators by stimulated monocytes. The components of tea tree oil that

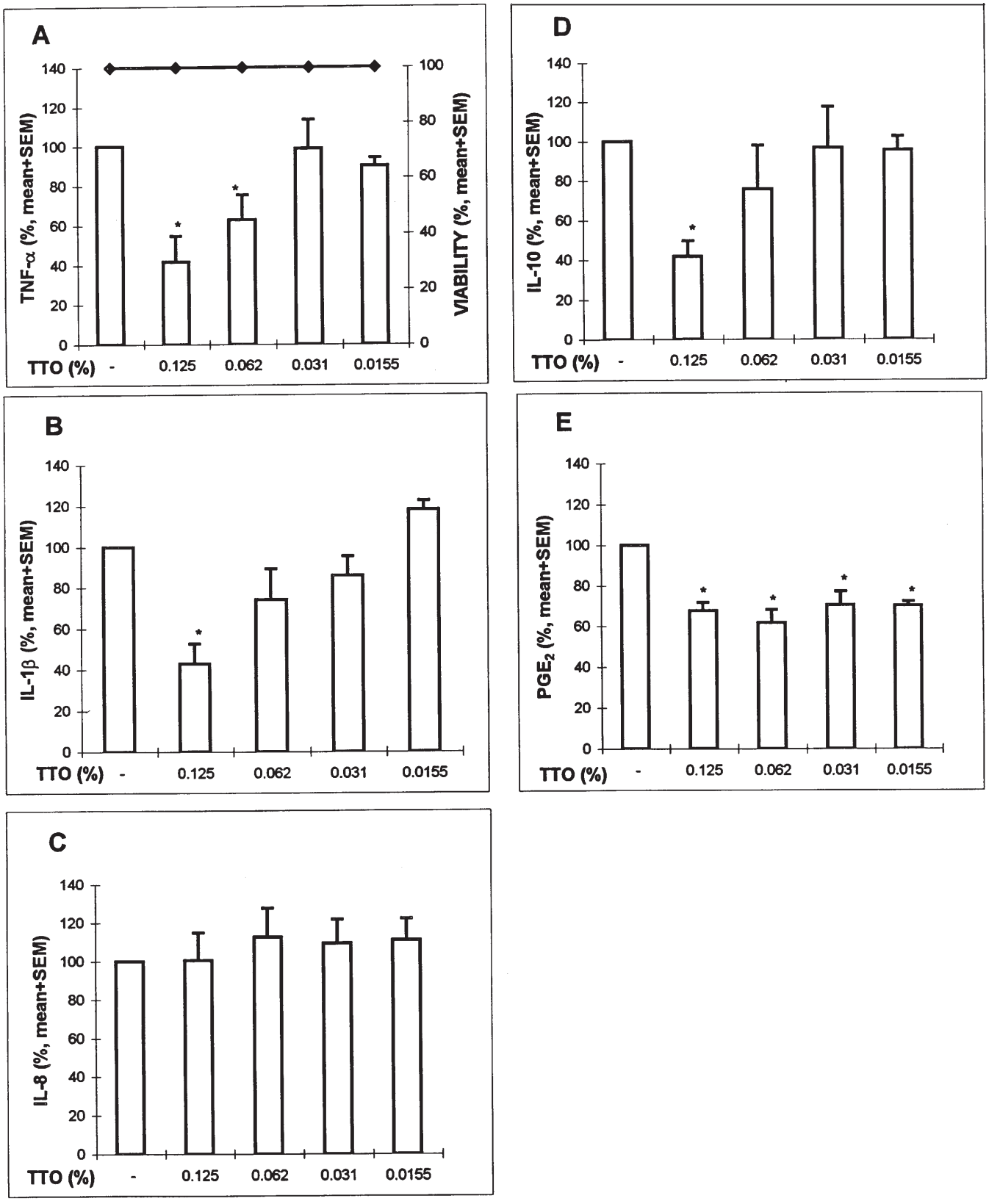

Fig. 2. The effect of tea tree oil diluted in polystyrene plastic tubes with serum-free medium on monocytes in culture for 40 h. Monocytes from 5 donors were incubated in triplicate under conditions allowing monocyte adherence for 40 h with LPS (500 ng/ml) and decreasing amounts of tea tree oil. The LPS-induced level was normalised to 100%; the mean result from each experiment was used to calculate the mean + SEM. Regulation of LPS-induced A. TNFα, B. IL-1β, C. IL-8, D. IL-10 and E. PGE_2 levels is shown by histograms. For A, the mean percentage + SEM (if sufficiently large) of viable cells in the cultures is shown by the line (right axis). An asterisk indicates a significant reduction in mediator production.

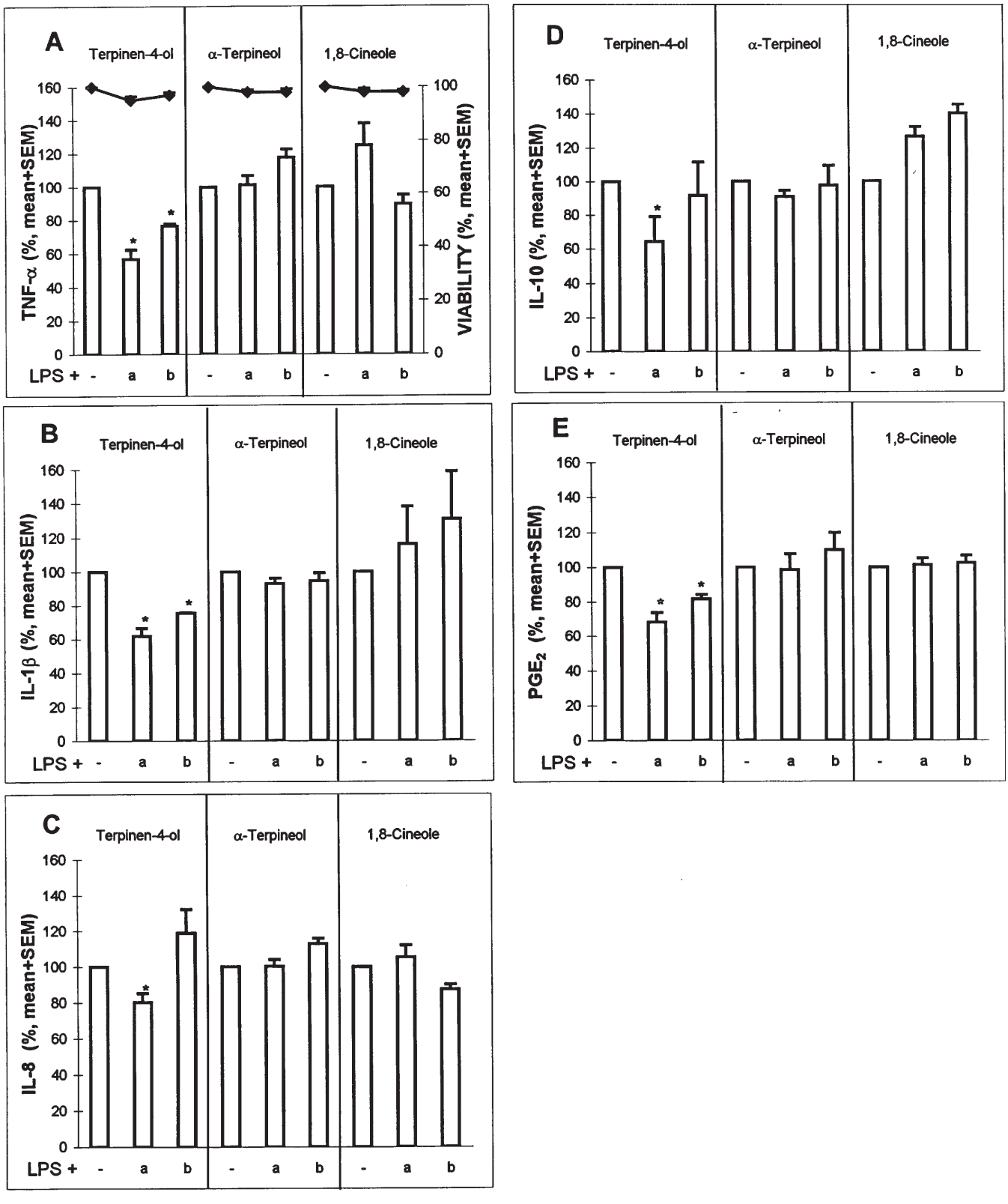

Fig. 3. The effect of the water-soluble components of tea tree oil diluted in glass tubes with serum-free medium on monocytes in culture for 40 h. Monocytes from 3 donors were incubated in triplicate under conditions allowing monocyte adherence for 40 h with LPS (500 ng/ml) and concentrations of terpinen-4-ol, α-terpineol and 1,8-cineole equivalent to those found in 0.125% (shown as a) and 0.062% (shown as b) tea tree oil. The LPS-induced level was normalised to 100%; the mean result from each experiment was used to calculate the mean + SEM. Regulation of LPS-induced A. TNFα, B. IL-1β, C. IL-8, D. IL-10 and E. PGE_2 levels is shown by histograms. For A, the mean percentage + SEM (if sufficiently large) of viable cells in the cultures is shown by the line (right axis). An asterisk indicates a significant reduction in mediator production.

Table 2. Induction of cytokines and PGE_2 by LPS-activated adherent human peripheral blood monocytes.

	Mediator production (mean ± SEM, ng/ml)	
	20 h (n = 10)	40 h (n = 5)
TNF-α	6.2 ± 1.8	6.8 ± 1.6
IL-1β	8.2 ± 1.8	8.1 ± 2.3
IL-8	250.8 ± 28.2	448.5 ± 58.0
IL-10	0.3 ± 0.1	0.6 ± 0.2
PGE_2	6.1 ± 0.8	6.7 ± 1.9

Table 3. Suppressive effect of the water-soluble components of tea tree oil on LPS-induced cytokine and PGE_2 production by adherent human peripheral blood monocytes.

	Water-soluble components of tea tree oil		Terpinen-4-ol	
	20 h (n = 10)	40 h (n = 5)	20 h (n = 6)	40 h (n = 3)
TNF-α	23%	58%	58%	43%
IL-1β	27%	57%	30%	38%
IL-8	19%	–	31%	20%
IL-10	–	58%	–	36%
PGE_2	–	32%	25%	32%

– = no significant decrease.
$P < 0.05$ for all changes shown.

partitioned into the culture medium when diluted in glass or polystyrene plastic tubes, in the absence or presence of 10% FCS, were determined. Table 1 shows that under the initial conditions of dilution in glass tubes with medium containing 10% FCS, the aqueous phase contained detectable levels of α-pinene, α-terpinene, p-cymene, 1,8-cineole, γ-terpinene, terpinen-4-ol and α-terpineol. With dilution in polystyrene plastic tubes with medium containing 10% FCS, the aqueous phase (which still contained significant toxic activity for monocytes) contained p-cymene, 1,8-cineole, γ-terpinene, terpinen-4-ol and α-terpineol. Using plastic tubes and serum-free diluting medium, three components of tea tree oil were detected in the aqueous phase, namely 1,8-cineole, terpinen-4-ol and α-terpineol, with terpinen-4-ol representing 84% of the recovered material. The identity and relative proportion of these components were identical to those in the aqueous phase when 500 mg tea tree oil was rapidly mixed with 50 ml water in a glass vessel.

Effects of terpinen-4-ol, α-terpineol and 1,8-cineole on monocyte mediator production

The data in Figure 2 and Table 3 suggested that water-soluble components of tea tree oil can suppress inflammatory mediator production by monocytes in vitro. The GC/MS analyses suggested that terpinen-4-ol, α-terpineol and 1,8-cineole were the components of tea tree oil that were partially soluble in tissue culture medium. It was therefore hypothesised that these three components were not toxic and could suppress inflammatory mediator production by monocytes activated with LPS in vitro.

The amounts of terpinen-4-ol, α-terpineol and 1,8-cineole investigated were calculated according to their concentration in a 0.125% solution of tea tree oil. A concentration equal to their level in a solution of 0.062% tea tree oil was also investigated. To maximise the amount of terpinen-4-ol, α-terpineol and 1,8-cineole that remained in the aqueous phase, dilutions were performed in glass tubes. As for the cultures of Figure 2 and Table 3, dilutions were performed in the absence of serum and monocytes were cultured in medium containing 1% FCS.

When monocytes were incubated with 0.052 and 0.026% terpinen-4-ol, 0.004 and 0.002% α-terpineol and 0.0025 and 0.0013% 1,8-cineole (concentrations equivalent to those in 0.125 and 0.062% tea tree oil, respectively), no toxicity for monocytes was detected (Fig. 3). After incubation for 20 h, 0.052% terpinen-4-ol significantly suppressed LPS-induced TNFα, IL-1β, IL-8 and PGE_2 production (Table 3). The suppression of LPS-induced IL-10 was not significant. After incubation for 40 h, the lower concentration of terpinen-4-ol significantly suppressed LPS-induced IL-1β and PGE_2, as well as TNFα (Fig. 3). After incubation for 20 or 40 h, the other water soluble components of tea tree oil, namely α-terpineol and 1,8-cineole, were without effect (Fig. 3).

Discussion

The potential for tea tree oil to have anti-inflammatory activity (as anecdotally reported) was investigated in vitro using activated human monocytes. However, when tea tree oil was emulsified in tissue culture medium containing 10% FCS in glass tubes and incubated at a dilution of 0.016% with adherent monocytes for 20 h, approximately 30% of the cells retained viability. Tea tree oil components toxic for human cells in culture have been reported previously. In a Swedish study [18], tea tree oil at concentrations of approximately 0.03% (300 µg/ml) and 0.05% (500 µg/ml) caused 50% killing of gingival epithelial cells and fibroblasts, respectively, after 24 h incubation. In another Australian study [19], the toxicity of tea tree oil and its main water-soluble components (terpinen-4-ol, α-terpineol and 1,8-cineole) on five human cell lines was examined after 4 and 24 h incubation. Susceptibility of the cell lines to the toxicity of tea tree oil varied with 50% killing after 4 h at a concentration of 0.28% for HeLa (epithelioid), K562 (chronic myelogenous leukaemia) and Hep G2 (hepatocellular carcinoma) cells. A concentration of 0.06% tea tree oil over 4 h caused 50% killing of CTVR-1 (B cell-derived from bone marrow of a patient with acute myeloid leukaemia) and Molt-4 (lymphoblastic leukaemia) cell lines. After 24 h, the IC_{50} for HeLa cells was 0.27%, for K562 cells 0.03%, CTVR-1 cells 0.03%, Molt-4 cells 0.03% and Hep G2 cells 0.002% [19]. Thus, monocytes cultured in vitro have a susceptibility to the toxic effects of tea tree oil similar to that of K562, CTVR-1 and Molt-4 cells. When tea tree oil was diluted to a concentration which was not toxic or minimally toxic to monocytes (0.008% or lower), there were some suggestions of reduced production of

inflammatory mediators. However, it was difficult to rule out the effects of lowered LPS-stimulated production of mediators by dying cells. In subsequent experiments, partitioning of tea tree oil components into aqueous and oil phases was encouraged with the oil components adhering to the polystyrene plastic tubes. Inclusion of 10% serum provided sufficient emulsifying activity such that tea tree oil was still cytotoxic to monocytes. A similar effect was seen if lower concentrations of serum were used in the presence of 0.001% Tween 20 (data not shown). Only when tea tree oil was diluted with serum-free medium in polystyrene plastic tubes were the components toxic for monocytes not dissolved. Analysis of the components of tea tree oil remaining in the culture medium suggested that either terpinen-4-ol (84% of compounds identified), α-terpineol (7% of compounds identified) or 1,8-cineole (3% of compounds identified) were regulatory for inflammatory mediator production by LPS-activated monocytes in vitro. Further studies suggested that terpinen-4-ol was responsible in large part for the regulatory effects of tea tree oil. The effects of terpinen-4-ol after 40 h closely paralleled the effects of tea tree oil as a whole, suggesting that if there were any other water-soluble anti-inflammatory components in tea tree oil, they were minor.

The plastic-non-adherent, water soluble components of a concentration of tea tree oil of 0.125% were examined in the experiments of Fig. 2 and Table 3. As it is recommended that a 100% solution of tea tree oil is applied to skin, this concentration represents approximately one thousandth of that used and is a level less than the water solubility of tea tree oil (1.6 g/l). The ability of tea tree oil components to reach the epidermal and dermal tissues and enter the systemic circulation has not been measured. However, tea tree oil contains several components known to enhance skin penetration of other compounds, e.g. 1,8-cineole [20], limonene [21], terpinen-4-ol [22] and α-terpineol [22]. The exact concentrations of the water soluble components of tea tree oil in the culture medium were not determined for each experiment but were assumed to be similar to those of Table 1 (84% terpinen-4-ol, 7% α-terpineol and 3% 1,8-cineole). To test the activity of terpinen-4-ol, α-terpineol and 1,8-cineole in isolation, calculations were made according to their concentration in whole tea tree oil and therefore their approximate level in tea tree oil solutions of 0.125%. Thus, in experiments in which the independent activity of the components was analysed, concentrations higher than in the aqueous phase of tea tree oil were examined. It should be noted that even at these higher concentrations, no toxicity was observed. 1,8-Cineole, the main component of eucalyptus oil (85%) was not regulatory in our study. We are uncertain why in a previous study it significantly suppressed LPS-induced TNFα production in vitro by monocytes at a concentration of 0.00001% [23] and following administration to asthmatic patients caused reduced leukotriene B4 and prostaglandin E_2 production by monocytes ex vivo [24]. Our study identified terpinen-4-ol, the major component of tea tree oil (>30%), as the component with the ability to suppress the production of TNFα, IL-1β, IL-8, IL-10 and PGE_2 by LPS-activated monocytes.

It was apparent from our study that all mediators released by activated monocytes are not similarly regulated by the water-soluble components of tea tree oil. In particular, little regulation of LPS-induced IL-8 production was detected at 20 or 40 h. TNFα and IL-1β production was regulated at 20 h by the water-soluble components in tea tree oil and this regulation was enhanced after 40 h incubation. In contrast, regulation of LPS-induced IL-10 and PGE_2 production was not detected until the later time point. This difference reflects, in part, the production of mediators with autocrine regulatory activity. The greater regulation of TNFα is significant as it is widely recognised that control of TNFα production/activity can limit further production of other inflammatory cytokines [25]. TNFα is regarded as central to the development and maintenance of chronic inflammation; this was evidenced by the ability of anti-TNFα antibodies to reduce inflammatory diseases such as rheumatoid arthritis [26].

Our study suggests that tea tree oil may potentially control inflammatory responses to foreign antigens in the skin. With application of tea tree oil to skin, toxicity would be limited and the anti-inflammatory water-soluble components may penetrate into the vascularised dermis and regulate inflammatory processes. This study supports the selection of superior trees for propagation after identification of productive clones which produce tea tree oil high in terpinen-4-ol [27]. Alternatively, an aqueous extract of tea tree oil which produces a fraction very high in terpinen-4-ol, may be used as an anti-inflammatory agent. As we are uncertain of the concentrations of terpinen-4-ol that may penetrate beyond the stratum corneum, the potential of tea tree oil as a topical anti-inflammatory agent will only be confirmed by documentation of a reduction of inflammatory cells and mediators in skin after application of tea tree oil.

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