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Anti-inflammatory effects of 1,8-cineole (eucalyptol) improve glucocorticoid effects in vitro: A novel approach of steroid-sparing add-on therapy for COPD and asthma?

Lisa Joy Juergens[a], Kurt Racké[b], Izabella Tuleta[a], Meinolf Stoeber[a], Uwe R. Juergens[a,*]

[a] Department of Internal Medicine II—Pneumology, Bonn University Hospital, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany
[b] Institute of Pharmacology & Toxicology, University of Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany

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ABSTRACT

Background and purpose: Add-on therapies with the monoterpene 1,8-cineole were shown to be effective in controlled studies, on asthma and chronic obstructive pulmonary diseases (COPD) We hypothesized, that 1,8-cineole (C) can improve steroid effects when combined with guideline medications (budesonide (BUD), formoterol (F)) for asthma and COPD.
Methods: Peripheral blood monocytes of 12 healthy volunteers were isolated and stimulated (10^5cells/ml) with LPS (10 μg/ml, 20 h) alone or in the presence of C, BUD, BUD + F and C+(BUD + F) in therapeutically relevant concentrations. Inflammatory mediators (IL-1β, IL-6, IL-8, TNF-α) were determined in supernatants by ELISA.
Results: 1,8-cineole (0.15–1.5 μM) concentration dependently inhibited the LPS induced cytokine releases (IL-6 > IL1β > IL-8 ≥ TNF-α) up to 100%; BUD inhibited the cytokine releases in lower concentrations (10^{-9}–10^{-5}M) and in a different sequence (IL-1β > TNF-α = IL-6 = IL-8) up to 80%. Relevant airway concentrations of 1,8-cineole (2×10^{-6}M) increased the inhibitory effects of BUD (10^{-9}M ≤ 28%). 1,8-cineole in concentrations ≥4×10^{-6}M inhibited already alone releases of all cytokines between 80 and 100% without additional effects of budesonide. BUD (10^{-11}M–10^{-9}M) + F (10^{-9}M) inhibited dose-dependently TNF-α and IL-1β releases up to 59 and 40%, respectively. Co-incubation with 1,8-cineole (4×10^{-6}M) enhanced the inhibition of TNF-α releases between 15 and 33% and the one of IL-1β releases between 14 and 44.5% ($p < 0.05$).
Conclusions: These results suggest a new mode of action of 1,8-cineole as non-steroidal, anti-inflammatory agent. Its potential to suppress airway inflammation and to improve the efficacy of inhaled steroids in COPD and asthma should be clinically further investigated.

1. Introduction

Eucalyptol (1,8-cineole) is known as the major ingredient of eucalyptus oil (80%)which has several drug qualities that have developed an increasing clinical interest. 1,8-cineole is a natural saturated monoterpene and is the active ingredient of the small gut soluble and in Germany registered medicine Soledum^R forte, that has been used for the symptomatic treatment of sinusitis and bronchitis due to its mucolytic, antimicrobial and spasmolytic properties. At least over the last 15 years there is an increasing evidence of its anti-inflammatory and antioxidant mode of action suggesting a causative rather than a pure symptomatic, secretolytic activity, as recently reviewed [1].

Mucus hypersecretion is the characteristic pathophysiological feature of acute and chronic upper and lower airway diseases. It is induced by an increased production of inflammatory mediators

Abbreviations: AQLQ, asthma quality of life questionnaire; BUD, budesonide; C, 1,8-cineole; COPD, chronic obstructive lung disease; COX-2, cyclooxygenase-2; ELISA, enzyme-linked immune-sorbent assays; FCS, fetal calf serum; F, formoterol; FEV1, forced expiratory volume in 1 second; HDAC-2, histone deacetylase-2; ICS, inhaled corticosteroids; IL, interleukin; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic receptor antagonist; LPS, lipopolysaccharide; PBM, peripheral blood monocytes; PGE2, prostaglandin E2; NF-κB, nuclear factor-kappaB; SGRQ, St. Georges respiratory questionnaire; Th1/2, T-helper 1/2 lymphocytes; TNF-α, tumor necrosis factor alpha; hTRPA1, human transient receptor potential cation channel, subfamily A, member 1 (transient receptor potential ankyrin 1); TRPM8, transient receptor potential cation channel subfamily M member 8.

* Corresponding author.
E-mail addresses: Uwe.Juergens@ukbonn.de, juergens_uwe@t-online.de (U.R. Juergens).

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and superoxide radicals in infect and allergic exacerbated or chronic airway diseases. So far, 1,8-cineole was believed to act as secretolytic/mucolytic agent rather than by the control of inflammatory mediators. But there is increasing evidence, that 1,8-cineole might control the causes of hypersecretion [2]. Thus, 1,8 cineole would not only act as a simple symptomatic drug. This was the initial rationale for the use of 1,8-cineole as a complementary therapy for respiratory tract diseases. And indeed hypersecretion was recently shown to be controlled by 1,8-cineole in rhinosinusitis by a reduction of mucin-filled goblet cells. Interestingly, this effect was related to the reduction of the MUC-2 gene expression and the attenuation of NF-κB-activity [2]. The up-regulation of mucin synthesis and related goblet cell hyperplasia has been reported to be associated with different intracellular signaling pathways involving NF-κB (TNF-α), Th1/2 cytokines (IL-17;IL-9, IL13), IL-1β and COX-2 metabolites (PGE_2) that also control inflammatory mediator production [3]. These results suggest different non-specific, anti-inflammatory modes of action of 1,8-cineole to control Th1/2-driven phenotypes in asthma and COPD [4]. In this context in vitro-studies with monocytes and unselected lymphocytes have earlier shown the inhibition of Th1- (IL1-β, TNF-α) and Th2- (IL-4, IL-5) cytokine production at therapeutic concentrations of 1,8-cineole [5]. Recently, an in vitro study using mouse primary splenocytes reported an extended modulation of Th1/Th2 cytokine secretions by 1,8-cineole and various other terpenoids [6]. The effects of in vitro-studies were confirmed in a preliminary clinical study (double blind, placebo controlled, randomized; n = 32) in patients with severe asthma who achieved a well tolerated reduction of prednisolone in the presence of 1,8-cineole and demonstrated an oral glucocorticoid-saving capacity of oral daily prednisolone dosage of −36% (3.75 mg) compared to placebo (−7%, 0.91 mg) [7].

Knowledge is presently increases rapidly regarding benefits and side effects of inhaled corticosteroids (ICS) [8]. ICS can lead in different COPD phenotypes [9] to a delayed lung function decline with a partial resistance [10]. We aimed to study the anti-inflammatory activity of the monoterpene 1,8-cineole as compared with ICS. We further determined the potency of 1,8-cinelole to strengthen effects of ICS and of long–acting β2-agonists (LABA) + ICS on the inflammatory mediator production in human monocytes *in vitro*. Our results provide the first evidence that 1,8-cineole improves the effects of glucocorticoids and interacts with guideline medication of F and BUD (LABA + ICS) for COPD and asthma in vitro. Therefore, 1,8-cineole might be suitable as add-on therapy to control systemic and peripheral lung inflammation while sparing glucocorticoid consumption.

2. Material and methods

2.1. Subjects

Twelve healthy volunteers, without either personal or family history of atopic reactions, gave their written informed consent to donate venous blood (≤50 mls). All participants were non-smokers and had not taken any medication during the preceding 12 weeks. Informed consent of the blood donors and The permission of ethical committee of Bonn University was obtained (18/1996).

2.2. Isolation of peripheral blood monocytes

Peripheral blood monocytes (PBMs) were isolated for each experiment from 50mls of EDTA-blood by density gradient (1.068 g/ml) centrifugation (NycoPrep, Axis-Shield, Oslo, Norway) as described earlier [11]. Platelet-free monocytes (>95%), purified by sequential centrifugation (50g/10 min) through autologous cell-free and platelet-free plasma, as assessed by light microscopy and a vitality of more than 98% according to trypan blue exclusion, were used for the experiments. The average yield of vital monocytes was $2.8 \pm 1.2 \times 10^6$ cells.

2.3. Cell cultures and cytokine induction

Monocytes were cultured in different experimental settings at 37 °C for 20 h in RPMI 1640 (Life Technologies, Eggenstein, Germany) supplemented with 10% FCS in 48-well plates (Costar, Tecnorama, Fernwald, Germany) in a humidified air atmosphere containing 5% CO_2, in the presence of 10 μg/ml lipopolysaccharide (LPS) (Sigma, Germany) for 20 h to stimulate the cytokine production of cells, as previously described [12]. Under these culture conditions monocytes (10^5/ml) released a higher amount of TNF-α (3300 ± 500 pg) compared to IL-1β (1700 ± 300 pg) into the culture supernatants. The same culture conditions yielded very high IL-6 and of IL-8 releases. Therefore lower cell densities (2×10^4/ml and 10^3/ml) were seeded to assure reproducibility of mediator measurements of IL-6 and IL-8, respectively, and to investigate inhibitory effects. The cells were incubated without and with 1,8-cineole (C, 10^{-9}–10^{-5}M, Cassella-med, Cologne, Germany). The inhibitory effects of 1,8-cineole and budesonide (BUD) (10^{-12}–10^{-5}M) alone or in combination (BUD + C) on the inflammatory mediators (TNF-α; IL-1ß, IL-6, IL-8) were determined. Further the effect of the combination of BUD and formoterol (BUD + F) (Sigma, Germany) on inflammatory mediator production was determined. Relevant airway concentrations of BUD (10^{-11},10^{-10},10^{-9}M) and a fixed concentration of F (10^{-9}M) were used in the combination studies with 1,8-cineole. For 1,8-cineole, lowest concentrations which had been shown to be effective alone (10^{-6}, 2×10^{-6}, 4×10^{-6}M) were chosen.

The test substances were dissolved in ethanol resulting in a final concentration of <0.1% ethanol in the cell culture medium. Cytotoxic effects of 1,8-cineole (10^{-5}M with or without BUD + F) could be clearly ruled out by tryphan blue exclusion (0.5%/5 min) and by LDH-activity before and after LPS-stimulation.

2.4. Quantification of TNF-α and cytokine production

In all experiments, the profile of inflammatory mediators (TNF-α, IL-1β, IL-6, IL-8) was assayed by specific enzyme-linked immuno-sorbent assay (ELISA) (SPI-BIO/Cayman, France) in the culture supernatants stored less than 6 weeks at −80 °C. According to the manufacturer, the antibodies used for all mediators measured (100%) cross-reacted with each other <0.01%. Repeatability of measurements including sample preparation and inter-assay variation varied by less than 10% and by around 10–20% considering cell isolation or variability in normal subjects. 1,8-cineole (10^{-4}M) did not interfere with the cytokine assay systems used as determined by adequate measurement of known mediator concentrations in the presence of the test substance.

2.5. Statistical analysis

A minimum of 4 experiments with 8–15 replicates each were undertaken for each condition. All results are expressed as the mean ± SEM as compared to the LPS-control. The Mann & Whitney U nonparametric test was used for comparison. Two-sided p values were considered significant if <0.05. All analyses were performed using the StatView (SAS Institute Inc., North Carolina, USA).