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Research Paper

1,8-Cineole induces relaxation in rat and guinea-pig airway smooth muscle

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Abstract

Objectives 1,8-Cineole is a monoterpene with anti-inflammatory, vascular and intestinal smooth muscle relaxant activity. We have evaluated the potential bronchodilatatory activity of this compound.

Methods 1,8-Cineole was tested against carbachol, histamine, K^+ 80 mM and ovalbumin-induced bronchial contractions in Wistar rat or guinea-pig tissues. Some of the guinea-pigs had been previously sensitized with an intramuscular injection of 5% (w/v) ovalbumin/saline solution. Control animals received 0.3 ml saline. In separate experimental groups the response to 1,8-cineole (1–30 mg/kg), phenoterol (0.05–5 mg/kg) or vehicle (0.3% Tween in saline) was studied.

Key findings 1,8-Cineole decreased, *in vivo*, rat bronchial resistance with similar efficacy as phenoterol (66.7 ± 3.2% vs 72.1 ± 5.3%). On the other hand, the maximal relaxant response to 1,8-cineole in carbachol-precontracted rat tracheas was 85.5 ± 5.7% (IC50 = 408.9 (328–5196) μg/ml) compared with 80.2 ± 4.8% (IC50 = 5.1 (4.3–6.1) μg/ml) with phenoterol. The addition of 1,8-cineole to guinea-pig tracheal rings tonically contracted with K^+ 80 mM induced a concentration-related relaxation. The maximal relaxation elicited by 1,8-cineole was 113.6 ± 11.7% (IC50 127.0 (115.9–139.2) μg/ml) compared with 129.7 ± 14.6% (IC50 0.13 (0.12–0.14) μg/ml) achieved after phenoterol administration. In addition, the incubation of tracheal rings with 1,8-cineole (100, 300 or 1000 μg/ml), 15 min before inducing phasic contractions with K^+ 80 mM, decreased the maximal amplitude of the contraction by 31.6 ± 4.6, 75.7 ± 2.7 and 92.2 ± 1.5%, respectively. In another set of experiments, neither the maximal response nor the IC50 for the 1,8-cineole-induced relaxation were different between normal and ovalbumin-sensitized tissues. Moreover, the relaxation of bronchial rings contracted after exposure to 1 μg/ml ovalbumin occurred at a faster rate in rings pre-incubated with 1,8-cineole when compared with rings pre-incubated with vehicle only (Tween 0.3%). Therefore, in the first minute after the antigen challenge, the tracheal tissue relaxed after the peak contraction by 6.5, 21.4 ($P < 0.05$ vs control) and 66.9% ($P < 0.05$ vs control) in the presence of 100, 300 or 1000 μg/ml 1,8-cineole, respectively.

Conclusions 1,8-Cineole relaxed rat and guinea-pig (nonsensitized and ovalbumin-sensitized) airway smooth muscle by a nonspecific mechanism.

Keywords airway smooth muscle; bronchodilatatory activity; 1,8-cineole; phenoterol

Introduction

1,8-Cineole is a terpenoid oxide that is a very common component of essential oils of aromatic species especially from the genus *Eucalyptus*. Some *Eucalyptus* species are used in folk medicine to treat airway disturbances. For example *E. globulus* is used in Mexico as an antitussive, to treat bronchitis, asthma and cold.[1] This compound has been used as a nasal decongestant and antitussive.[2] 1,8-Cineole has also been used to treat bronchitis, sinusitis and chronic rhinitis and asthma.[3,4] A standardized phytotherapeutic extract consisting of three monoterpenes, including 1,8-cineole, was shown to induce rapid onset of regression of the signs and symptoms (including bronchial hyperreactivity) of acute bronchitis in humans,

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with similar efficacy when compared with patients treated with cefuroxime or ambroxol.[5] Furthermore, the inhibitory effects of 1,8-cineole over the production of inflammatory mediators may also translate into clinically relevant anti-inflammatory efficacy. Recent studies have shown, for example, that 1,8-cineole induced a clinically relevant and statistically significant amelioration in the symptoms of acute nonpurulent rhinosinusitis.[4] In another clinical trial, the majority of patients remained stable with chronic asthma after receiving oral 1,8-cineole (200 mg, three times per day), despite a mean reduction in the administration of oral steroids. This was in sharp contrast to the placebo group, in which patients were not able to tolerate any decrease of oral steroids. This was the first clinically relevant report of the anti-inflammatory activity of 1,8-cineole in bronchial asthma.[6]

In a single-blind study involving patients with mild and moderate asthma, additional therapy with 1,8-cineole over three days improved lung function and suppressed ex-vivo stimulated inflammatory mediator production in short-term cultures of peripheral monocytes. The activity was equivalent to prednisolone.[3]

Some pharmacological effects of 1,8-cineole were studied by Santos[7] in mice and rats. The compound was demonstrated to have low toxicity, to be gastroprotective, analgesic and anti-inflammatory. The reproduction of mice was not affected by this compound when administered by the oral route to pregnant mice after fertilization.[7] This compound was also shown to induce relaxation of the vascular and intestinal smooth muscle, and therefore may also have a relaxant activity on the airway smooth muscle.[8,9] Besides its smooth muscle relaxant activity, 1,8-cineole induced negative inotropic effect in rat cardiac tissues and blocked rat sciatic nerve excitability.[10,11]

1,8-Cineole was demonstrated to block the phasic component of the contraction induced by a high-potassium solution in guinea-pig trachea.[12] Nevertheless, no further investigation was performed to study the effects of this compound in the airway smooth muscle contractility of normal or antigen-sensitized animals.

Additionally, 1,8-cineole revealed a steroid-like suppression of arachidonic acid metabolism and cytokine production *in vitro*.[13] Moreover, 1,8-cineole inhibited monocyte inflammatory mediator production with a magnitude of effect comparable with that of budesonide, a potent synthetic corticosteroid.[14] Further controlled studies revealed a significant improvement in lung function tests.[15]

Recently it has been noticed that there has been a significant increase in the number of patients with asthma and other allergic respiratory diseases.[16,17] Conventional bronchodilator treatment with adrenergic β_2-agonists has some side effects. Recent studies have shown a small reduction in the bronchodilator effect during treatment with long-acting β-agonists. The downregulation of these airway β_2-receptors may also explain the phenomenon of rebound bronchoconstriction on treatment withdrawal.[18]

In this study we have shown that 1,8-cineole relaxed both normal and ovalbumin-sensitized guinea-pig airway smooth muscle. The anti-inflammatory effect of 1,8-cineole associated to its airway smooth muscle relaxant activity may point to a potential benefit of using this product to treat or prevent bronchial hyperreactivity.

Materials and Methods

Animals

Wistar rats, from both sexes (250–300 g), or male guinea-pigs (400–500 g) were obtained from the animal vivarium of the State University of Ceara. The animals were treated according to the ethical proceedings from the Brazilian College of Animal Experimentation (COBEA). The protocols were approved by the Committee for Ethics in Research of the State University of Ceará. For sensitization of guinea-pigs the animals were injected intramuscularly with a 5% (w/v) ovalbumin/saline solution into each thigh on days one and four. Control animals received 0.3 ml saline into each thigh on days one and four. The guinea-pigs were used for experiments at day 25.

Tracheobronchial resistance *in vivo*

This procedure followed the original descriptions of Konzett and Roessler.[19] Briefly, Wistar rats from both sexes (250–300 g) were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and kept at 37°C by means of a heat pad. The right common carotid artery and internal jugular vein were dissected and cannulated with polyethylene catheters, one for the measurement of arterial pressure and the other for intravenous injection of drugs. The arterial blood pressure was continuously recorded by means of a Staham P23 pressure transducer (Gould, Oxnard, California, US) coupled to a two-channel desk physiograph (Gemini 7070 Ugo Basile, Varese, Italy). A stainless steel tube was inserted in the trachea and coupled to a rodent ventilator (Rodent Ventilator 7025 Ugo Basile) calibrated to give 1 ml/100 g tidal volume and 40 ventilations/min. The animals received a single injection of pancuronium (5 mg/kg) to avoid interference of the respiratory muscles with the artificial ventilation. A bronchospasm transducer was coupled to the arm side of the system (model 7020 Ugo Basile) and attached to a multifunctional pre-amplifier (Ugo Basile, model 7082) and a two-channel polygraph (Gemini 7070 Ugo Basile). After 15-min equilibration, a single dose of carbachol (30 μg/kg) was injected to induce a sustained bronchoconstriction. In separate experimental groups the response to 1,8-cineole (1–30 mg/kg), phenoterol (0.05–5 mg/kg) or vehicle (0.3% Tween in saline) was studied in established carbachol-induced bronchoconstrictions.

Guinea-pig and rat tracheal chain and guinea-pig bronchial rings

Male Wistar rats or Albino male guinea-pigs were killed under sodium pentobarbital anaesthesia and the trachea was carefully dissected and excised out. The experimental procedure followed the original description of Castillo and De Beer.[20] Briefly, trachea was cut transversely between the segments of cartilage to give six tracheal rings that were tied together. Bronchial rings, used for experimental antigenic challenge, were obtained from the hilar bronchi and cleaned of the parenchyma. The tissues were attached to an isometric transducer (F-60 Narco Biosystems, Houston, Texas, US) connected to a four-channel polygraph (Narco Biosystem) and kept in solution (Krebs–Henseleit solution; 37°C; pH 7.4; gassed with 95% O_2 in 5% CO_2) in a 5-ml organ bath chamber.

The Krebs–Henseleit solution had the following composition (mM): NaCl 114.6, KCl 4.96, $MgSO_4$ 1.3, $CaCl_2$ 2.0, NaH_2PO_4 1.23, $NaHCO_3$ 25, and 3.6 glucose. The high-potassium solution (K^+ 80 mM) was made by equimolar replacement of NaCl by KCl in the Krebs–Henseleit solution. After 1-h equilibration and washing out every 15 min, in independent sets of experiments, the rat trachea was precontracted with 1 μM carbachol and the guinea-pig trachea with K^+ 80 mM solution. Thereafter, in the plateau phase of tonic contractions 1,8-cineole (1–3000 μg/ml), phenoterol (0.1–100 μg/ml) or equal volumes of vehicle (Tween 0.3% in saline) was administered to construct concentration–response curves. In another set of experiments, 1,8-cineole (30, 100, 300 or 1000 μg/ml) was added to the organ baths 15 min before the induction of phasic contractions with K^+ 80 mM. The phasic component was considered at the peak deflection after 5–10 s exposure to the K^+ 80 mM solution. The amplitude of phasic contractions were compared in tissues pretreated with 1,8-cineole or vehicle. Thereafter, in other independent sets of experiments the concentration–response curve to 1,8-cineole (1–3000 μg/ml), phenoterol (0.1–100 μg/ml) or equal volumes of vehicle (Tween 0.3% in saline) was studied in both normal or ovalbumin-sensitized guinea-pig trachea precontracted with histamine (10 μM). Finally, the effects of 30-min incubation of bronchial rings with 1,8-cineole (100, 300 or 1000 μg/ml) on the contraction induced by ovalbumin (1 μg/ml) in bronchial rings obtained from previously sensitized guinea-pigs were studied.

Statistical analysis

The data were expressed as mean ± SEM of seven experiments. The statistical differences were verified by using analysis of variance followed by the correction of Tukey–Kramer with the significance level set at 5%. The IC50 values, defined here as the concentration that induced 50% of the maximal response, were calculated by using the GraphPad Prism 3.00 software and were expressed as mean ± 95% confidence interval.

Results

On anaesthetized rats challenged with carbachol, 1,8-cineole decreased the tracheobronchial resistance *in vivo* in a measured dose-related manner. The maximal response (R_{max}) achieved with 1,8-cineole was not different when compared with the response obtained with phenoterol (66.7 ± 3.2 vs 72.1 ± 5.3%; $P > 0.05$; $n = 7$) (Figure 1).

To investigate whether 1,8-cineole directly relaxed the airway smooth muscle we tested its effects on isolated rat and guinea-pig tracheal chains. This compound relaxed rat tracheal chains precontracted with 1 μM carbachol (2.2 ± 0.19 g) in a dose-dependent manner. The maximal relaxation of the airway smooth muscle to 1,8-cineole was 85.5 ± 5.7% compared with 80.2 ± 4.8% achieved with phenoterol (Figure 2). Nevertheless, phenoterol was approximately 80-fold more potent than 1,8-cineole in this experiment. The calculated IC50 with 95% confidence interval for phenoterol was 5.1 (4.3–6.1) μg/ml compared with 408.9 (328–5196) μg/ml calculated for 1,8-cineole.

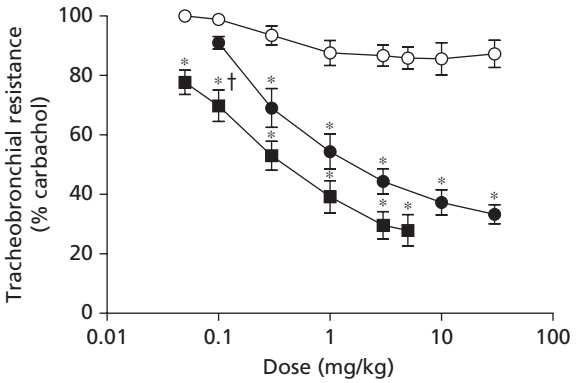

Figure 1 Effect of 1,8-cineole on tracheobronchial resistance in anaesthetized rats. The drugs were injected as a bolus through the jugular vein and vehicle (0.3% Tween in saline, v/v) was injected isovolumetrically by the same route. *$P < 0.05$ analysis of variance followed by Tukey (drugs vs vehicle (○). †$P < 0.05$ (phenoterol (■) vs 1,8-cineole (●)). The data are expressed as mean ± SEM of five experiments.

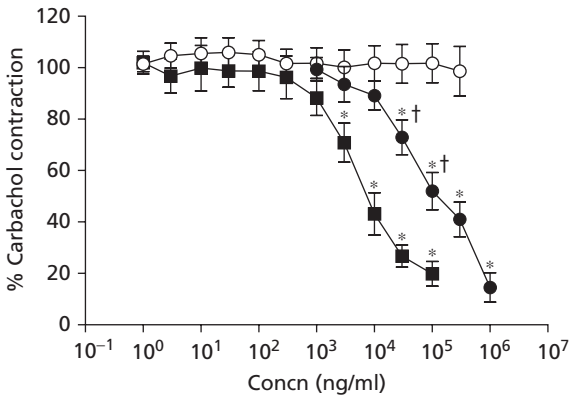

Figure 2 Effect of 1,8-cineole on rat tracheal rings precontracted with carbachol. Tracheal rings were precontracted with 1 μM carbachol. The effect of 1,8-cineole (●) was compared with the β_2-agonist phenoterol (■). *$P < 0.05$ analysis of variance followed by Tukey (drugs vs vehicle (○). †$P < 0.05$ analysis of variance followed by Tukey (1,8-cineole vs phenoterol). The data are expressed as mean ± SEM of six experiments.

The addition of 1,8-cineole to guinea-pig tracheal rings tonically contracted (2.8 ± 0.46 g) by a high-potassium solution (K^+ 80 mM) induced a concentration-related relaxation (Figure 3). The maximal relaxation, expressed as a percentage of the K^+-induced contraction, elicited by 1,8-cineole was 113.6 ± 11.7% (IC50 127.0 (115.9–139.2) μg/ml) compared with 129.7 ± 14.6% (IC50 0.13 (0.12–0.14) μg/ml) achieved after phenoterol administration. In addition, the incubation of tracheal rings with 1,8-cineole (100, 300 or 1000 μg/ml) 15 min before inducing phasic contractions (3.5 ± 0.5 g) with K^+ 80 mM decreased the maximal amplitude of the contraction by 31.6 ± 4.6, 75.7 ± 2.7 and 92.2 ± 1.5%, respectively (Figure 4).

Moreover, the tonic contraction (2.4 ± 0.3 g) induced by 10 μM histamine was completely relaxed by 1,8-cineole in nonsensitized guinea-pig tracheas. After washing out the tissue (1 h), the tracheas were still able to contract after a

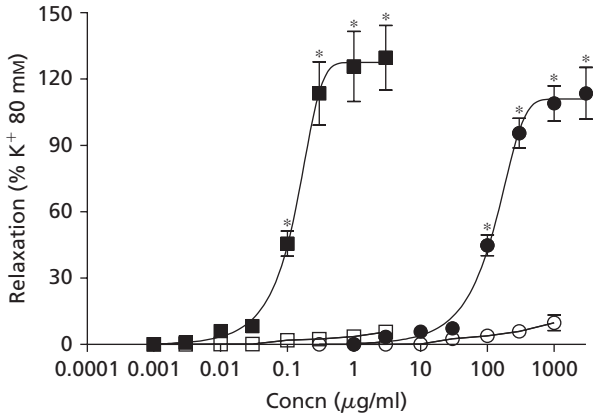

Figure 3 Effect of 1,8-cineole on the tonic component of the K^+ 80 mM-induced contraction in guinea-pig tracheal rings. The effect of 1,8-cineole (●) was compared with the β_2-agonist phenoterol (■). *$P < 0.05$ analysis of variance followed by Tukey (1,8-cineole vs vehicle). Vehicle plus 1,8-cineole (○); vehicle plus phenoterol (□). The data are expressed as mean ± SEM of five experiments.

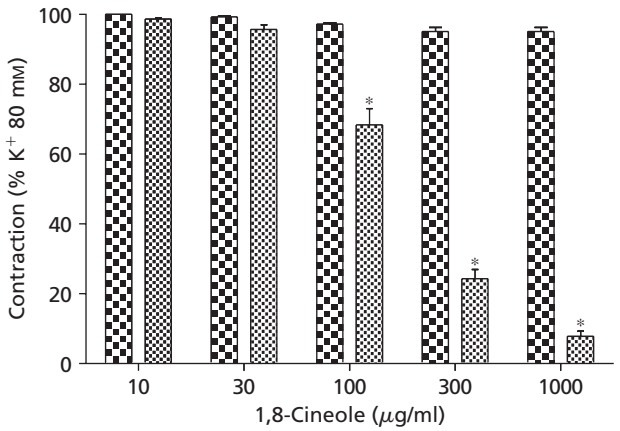

Figure 4 Effect of 1,8-cineole on the phasic component of the K^+ 80 mM-induced contraction in guinea-pig tracheal rings. 1,8-Cineole was added 15 min before the induction of contractions. The vehicle was applied isovolumetrically. *$P < 0.05$ analysis of variance followed by Tukey (1,8-cineole vs vehicle). The data are expressed as mean ± SEM of four experiments. K^+ 80 mM plus vehicle, columns of squares; K^+ 80 mM plus 1,8-cineole, cross-hatched column.

new histamine challenge and were also responsive to phenoterol, showing functional recovery after the 1,8-cineole concentration–response curve (Figure 5). The same pattern was observed in ovalbumin-sensitized guinea-pig tracheas when compared with nonsensitized tissues. Neither the maximal response nor the IC50 for the 1,8-cineole-induced relaxation were different between normal and sensitized tissues (Figure 6). The maximal relaxation achieved after 1,8-cineole administration was 108.0 ± 9.6% (IC50 150.3 (124–182.1) μg/ml) in normal tissues vs 102.4 ± 11.7 (IC50 217.8 (148.6–319.3) μg/ml) in ovalbumin-sensitized guinea-pig tracheas. Similarly, the potency of phenoterol for inducing relaxation in ovalbumin-sensitized tracheas

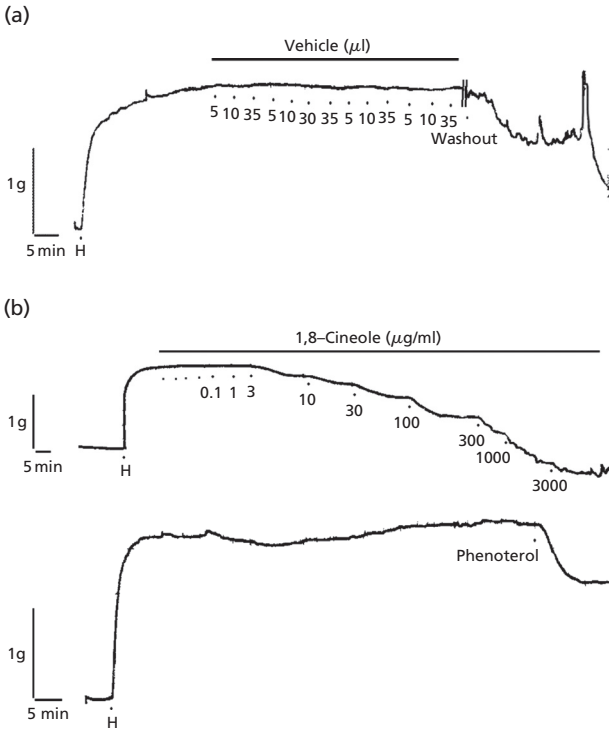

Figure 5 Typical physiographic recordings of the effect of the vehicle or 1,8-cineole in the plateau phase of the tonic contraction induced by histamine in nonsensitized guinea-pig tracheal chains. (a) Vehicle (Tween 0.3% in saline; v/v) or (b) 1,8-cineole. After washing out 1,8-cineole, the preparation was able to contract to histamine (10 μM; H) and relax to phenoterol (0.1 μg/ml), showing the functional recovery of the tissue.

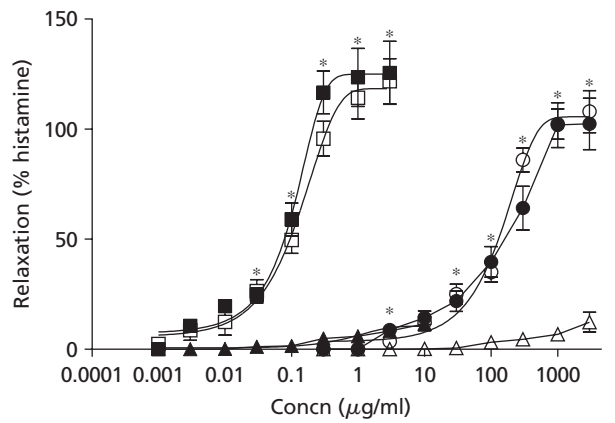

Figure 6 Effect of 1,8-cineole on normal or ovalbumin-sensitized guinea-pig tracheal rings precontracted with histamine. Tracheal rings were precontracted with 10 μM histamine. The compounds tested were applied cumulatively in the bath at increasing concentrations. Vehicle was isovolumetrically applied as a negative control. *$P < 0.05$ analysis of variance followed by Tukey (drugs vs vehicle). The data are expressed as mean ± SEM of six experiments. Tween 0.3%, △; saline, ▲; 1,8-cineole, ○; 1,8-cineole plus ovalbumin, ●; phenoterol, □; phenoterol plus ovalbumin, ■.

precontracted with histamine was similar to the value obtained in normal tracheas (Figure 6). The IC50 varied from 0.14 (0.09–0.19) μg/ml in control tissues to 0.11 (0.09–0.13) μg/ml in ovalbumin-sensitized tissues.

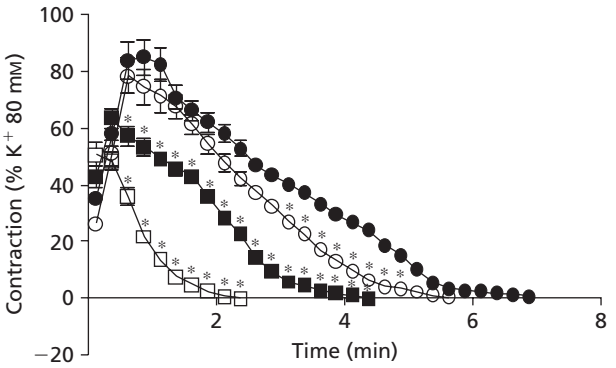

Figure 7 Effect of 1,8-cineole on the Schultz-Dale reaction induced contraction in guinea-pig tracheal rings. 1,8-Cineole was applied 30 min before the addition of ovalbumin (1 μg/ml). The vehicle was applied isovolumetrically. *$P < 0.05$ analysis of variance followed by Tukey (1,8-cineole vs vehicle). The data are expressed as mean ± SEM of five experiments. Ovalbumin plus vehicle, ●; ovalbumin plus 1,8-cineole 100 μg/ml, ○; ovalbumin plus 1,8-cineole 300 μg/ml, ■; ovalbumin plus 1,8-cineole 1000 μg/ml, □.

The guinea-pig bronchial rings contracted in response to K^+ 80 mM, as an internal standard, with an active force of 1.70 ± 0.13 g ($n = 20$). The 30-min pre-incubation of bronchial rings from sensitized guinea-pigs with 1,8-cineole before 1 μg/ml ovalbumin challenge decreased the maximal developed tension (ovalbumin 1.46 ± 0.10 g; $n = 5$) only at 1000 μg/ml (ovalbumin+1,8-cineole (1000 μg/ml) 0.86 ± 0.12 g; $n = 5$; $P < 0.05$ vs ovalbumin alone). In addition, the relaxation of the bronchial rings occurred at a faster rate in the 1,8-cineole pre-incubated rings when compared with rings pre-incubated with the vehicle (Tween 0.3%) (Figure 7). In the first minute, ovalbumin-induced contraction relaxed by 6.5, 21.4 ($P < 0.05$ vs control) and 66.9% ($P < 0.05$ vs control) in the presence of 100, 300 or 1000 μg/ml 1,8-cineole, respectively. At the same time, the relaxation observed in the control group, pretreated with Tween 0.3% in saline (v/v), was only 2.2%.

Discussion

1,8-Cineole was shown to induce a rapid onset of bronchodilation *in vivo* after a sustained bronchospasm induced by carbachol challenge. This compound also directly relaxed, *in vitro*, carbachol, high-potassium and histamine precontracted airway smooth muscle. Since 1,8-cineole relaxed tracheal smooth muscle contracted with different agonist with similar potency it was probable that this effect was nonspecific for membrane receptors. In this regard, Magalhães *et al.*[9] have shown that 1,8-cineole relaxed guinea-pig ileum precontracted with acetylcholine, histamine or high-potassium solutions with similar potency, probably by a nonspecific interference with the excitation–contraction coupling mechanism.

The relaxation of high-potassium (K^+ 80 mM) precontracted tracheal rings induced by 1,8-cineole suggested that this compound was unlikely to relax airway smooth muscle by a K^+-channel opening mechanism, since in this condition, i.e. K^+-contracture, further alterations in potassium conductance have little influence in muscle tonus.

The inhibition of phasic contractions induced by the high-potassium solution suggested that 1,8-cineole may have had an antagonist action on the calcium transmembrane influx or in its intracellular action as a second messenger. This study showed similar inhibition of phasic high-potassium contractions in accordance with the results presented by Coelho-de-Souza *et al.*[12]. In addition, Soares *et al.*[10] have shown that 1,8-cineole could induce negative inotropic effect on rat cardiac tissues and concluded that this compound did not interfere with the sarcoplasmic reticulum function but rather blocked calcium influx through the membrane. Therefore, the relaxation induced by 1,8-cineole in the rat and guinea-pig trachea may have been due to its negative interference on calcium flux across the cell membrane.

The relaxation induced by 1,8-cineole may also have had a neurogenic component since this compound, used in concentrations similar to that used in this study, was shown to block peak to peak amplitude of the neuronal action potential as well as the conduction velocity of the isolated rat sciatic nerve.[11]

The prompt reversion of both contractile and relaxing function of the trachea after washing out 1,8-cineole from tissue baths may imply that this compound had low toxicity to the smooth muscle of trachea, at least *in vitro*. This compound was also shown to have low toxicity when applied orally to rats in graded doses up to 1 g/kg body weight, when no signs of acute toxicity were noticed (data not shown).

On the other hand, the increased rate of relaxation of ovalbumin-challenged tissues in the presence of 1,8-cineole may have been the result of both the smooth muscle relaxant activity of 1,8-cineole and the inhibition of the release of some contractile mediator upon ovalbumin challenge due to the anti-inflammatory properties of this compound. For instance, Juergens *et al.*[3] have shown that 1,8-cineole inhibited in a dose-related fashion the production of pro-inflammatory cytokines such as interleukin-1-β, tumour necrosis factor-α, thromboxane B_2 and leukotriene B_4.

This anti-inflammatory activity was also demonstrated by Santos and Rao[21] as its gastroprotective effect against ethanol-induced gastric mucosal damage.[22] This later activity was associated to an anti-oxidant action of 1,8-cineole and to its inhibitory effect on lipoxygenase activity and leukotriene formation.[22] Leukotrienes are believed to be associated with histamine, one of the major mediator responsible for the anaphylactic contraction of the airways from in humans and guinea-pigs.[23–25]

Conclusions

1,8-Cineole directly relaxed airway smooth muscle of rats and guinea-pigs by a nonspecific mechanism, probably related to the blockage of calcium influx across the membrane. The compound also increased the rate of relaxation of ovalbumin-sensitized bronchial rings after a Schultz-Dale-like reaction. This effect was probably due to the known anti-inflammatory properties of this compound as an inhibitor of the generation of leukotrienes.

This has been the first report demonstrating the direct bronchodilatatory activity of 1,8-cineole by a nonspecific mechanism and further studies are necessary to test its efficacy and relative safety in conditions related to airway tract inflammation associated with bronchospasm.

Acknowledgements

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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