



1,8-cineole (eucalyptol) ameliorates cerulein-induced acute pancreatitis via modulation of cytokines, oxidative stress and NF- κ B activity in mice

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ABSTRACT

Aims: Acute pancreatitis (AP) is an inflammatory condition wherein pro-inflammatory mediators, oxidative stress, and NF- κ B signaling play a key role. Currently, no specific therapy exists and treatment is mainly supportive and targeted to prevent local pancreatic injury and systemic inflammatory complications. This study was aimed to examine whether 1,8-cineole, a plant monoterpene with antioxidant and anti-inflammatory properties could ameliorate cerulein-induced acute pancreatitis.

Main methods: AP was induced in Swiss mice by six one hourly injections of cerulein (50 μ g/kg, i.p.). 1,8-cineole (100, 200 and 400 mg/kg, p.o.) was administered 1 h prior to first cerulein injection, keeping vehicle and thalidomide treated groups as controls. Blood samples were taken 6-h later to determine serum levels of amylase and lipase, and cytokines. The pancreas was removed for morphological examination, myeloperoxidase (MPO) and malondialdehyde (MDA) assays, reduced glutathione (GSH) levels, and for nuclear factor (NF)- κ B immunostaining. **Key findings:** 1,8-cineole effectively reduced the cerulein-induced histological damage, pancreatic edema and NF- κ B expression, levels of MPO activity and MDA, and replenished the GSH depletion. Cerulein increased serum levels of amylase and lipase, and pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 were also decreased by 1,8-cineole pretreatment, similar to thalidomide, a TNF- α inhibitor. The anti-inflammatory IL-10 cytokine level was, however, enhanced by 1,8-cineole.

Significance: These findings indicate that 1,8-cineole can attenuate cerulein-induced AP via an anti-inflammatory mechanism and by combating oxidative stress. Further studies are needed to clearly elucidate its benefits in patients on acute pancreatitis.

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Introduction

Acute pancreatitis (AP) is a morbid inflammatory condition of pancreas with no specific treatment available. The incidence of pancreatitis has increased in general population during the past two decades (Frossard et al., 2008), much of which may be due to cholelithiasis, alcohol abuse, hypertriglyceridaemia, hyperparathyroidism, pancreatic malignancy, obesity, endoscopic retrograde cholangiopancreatography (ERCP), trauma, infectious agents, drugs, autoimmunity, and hereditary (Frossard et al., 2008; Zamri and Razman, 2012). The pathogenesis of pancreatitis remains still obscure and multifactorial. The disease is

believed to initiate in acinar cells with the intra-acinar cell activation of pancreatic proenzymes and NF- κ B (Huang et al., 2013) leading to local and systemic overwhelming production of inflammatory mediators and early organ dysfunction (Kylänpää et al., 2012) with death of acinar cells through both necrosis and apoptosis. Participation of reactive oxygen species and a role for oxidative stress play an important role in the pathogenesis of AP in animal models and in humans and a significantly greater serum concentrations of sulfhydryl groups and thiobarbituric acid reactive substances (TBARS) were observed in the early phase of human AP (Dziurkowska-Marek et al., 2004; Leung and Chan, 2009). Agents that prevent oxidative stress, inflammation, and acinar cell injury during the early phase of acute pancreatitis may arrest the pathologic progression to severe pancreatitis that leads to significant morbidity and mortality. Current therapies included the use of antisecretory agents, protease inhibitors, antioxidants, immunomodulators, non-steroidal anti-inflammatory medications, and prophylactic antibiotics (Easler et al., 2012). A few of

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these therapies have demonstrated promise in significantly altering the progression of this disease and the search for more effective therapy continues.

1,8-cineole (eucalyptol), is a major monoterpenoid present in many plant volatile/essential oils, principally the *Rosemarinus* and *Eucalyptus* species (Elaissi et al., 2012; Tschiggerl and Bucar, 2010). It is often employed by the pharmaceutical industry in drug formulations, as a percutaneous penetration enhancer and for its decongestant and anti-tussive effects and in aromatherapy as a skin stimulant (Santos and Rao, 2000). Our previous studies identified 1,8-cineole as a substance with analgesic and anti-inflammatory, and hepatoprotective properties and to attenuate the colonic damage in rats on acute trinitrobenzene sulfonic acid-induced colitis, an animal model of inflammatory bowel disease of humans (Santos and Rao, 2000; Santos et al., 2004). Few other studies described 1,8-cineole as strong inhibitor of TNF- α and IL-1 β controlling airway mucus hypersecretion, suggesting its usefulness in asthma, sinusitis and chronic obstructive pulmonary disease (Juergens et al., 2003, 2004), and as a compound possessing lipid-lowering, antioxidant and anti-inflammatory properties in hypercholesterolemic zebrafish (Cho, 2012). These observations suggest that 1,8-cineole may have beneficial effects in the management of acute pancreatitis. Further, a recent study reported on the protective effects of α -pinene yet another monoterpene present in plant essential oils, in mice on cerulein-induced acute pancreatitis (Bae et al., 2012). Therefore, this study was aimed to evaluate the protective effect of 1,8-cineole in a murine model of cerulein-induced pancreatitis that presents the characteristics of human pancreatitis in its pathology.

Materials and methods

Animals

Adult male Swiss mice weighing 25–30 g obtained from the central animal house of this university were used. They were housed in polypropylene cages at 23 ± 2 °C under standard environmental conditions (humidity 55%–60% and 12 h light–dark photoperiod) and had free access to pellet diet (purina chow) and tap water. The animals were fasted for 12 h before beginning the experiments and eight animals were included in each group. Experimental protocols were approved by the Institutional Committee on Care and Use of Animals for Experimentation (No. 30/12) in accordance with the guidelines of the National Institute of Health guide for the care and use of laboratory animals.

Drugs

1,8-cineole and cerulein were purchased from Sigma-Aldrich (St Louis, MO, USA) and thalidomide was purchased from Fumed (Belo Horizonte, MG, Brazil). 1,8-cineole was dissolved in normal saline with 2% Tween 80 whereas all other drugs were dissolved in normal saline (0.9% NaCl) immediately before use. All drugs were administered at a volume of 10 mL/kg body weight.

Cerulein-induced acute pancreatitis

Acute pancreatitis was induced by five intraperitoneal injections of cerulein (50 μ g/kg) at intervals of 1 h (Malleo et al., 2008). The normal control mice were given intraperitoneal saline (0.9% NaCl) solution instead of cerulein injections. Six hours after the last injection of cerulein or saline, mice were anesthetized with pentobarbital (40 mg/kg, i.p.), blood samples were drawn and the animals were exsanguinated, and the pancreas was quickly removed and frozen at -70 °C until use. 1,8-cineole (100, 200 and 400 mg/kg), thalidomide (200 mg/kg) and vehicle (2% Tween 80 in 0.9% NaCl) were administered orally 1 h prior to the first administration of cerulein.

Determination of pancreatic edema

The pancreatic weight/body weight ratio was evaluated as an estimate of the degree of pancreatic edema (Szabolcs et al., 2006).

Determination of serum parameters

Blood samples were collected 6 h after the last cerulein administration and then centrifuged at $3000 \times g$ for 10 min at 4 °C. The serum amylase and lipase levels were determined with a colorimetric method using a commercial kit for amylase (Labtest, MinasGerais, Brazil) and lipase (Bioclin, Minas Gerais) and were expressed as U/L and U/dL, respectively. Tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, and IL-10 levels were estimated using the commercially available immunoassay ELISA kits (Quantikine, R&D System, Minneapolis, MN, USA), according to the manufacturer's instructions. The results were expressed as pg/mL.

Pancreatic myeloperoxidase

The pancreatic myeloperoxidase (MPO) activity was determined according to Bradley et al. (1982). Samples of pancreatic tissue were homogenized in 50 mM of phosphate buffer (pH 6), containing 0.5% hexadecyltrimethylammoniumbromide (HTAB). The samples were freeze-thawed thrice with sonication between cycles and then the samples were centrifuged at $40,000 \times g$ for 15 min. Aliquots of supernatant were added to the reaction mixture containing 0.167 mg/mL of *o*-dianisidine and 0.0005% H₂O₂ solution, which were prepared in 50 mM of phosphate buffer. The resulting change in absorbance at 460 nm was measured spectrophotometrically for 5 min. One unit of MPO activity was defined as that degrading 1 mmol of peroxide per min at 25 °C. The activity was expressed as U/mg of tissue.

Pancreatic malondialdehyde

The concentration of pancreatic lipid peroxidation was determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test (Agar et al., 1999). The pancreatic tissue was homogenized in KCl 0.15 M (pH 7.4). The homogenate was maintained in a water bath for 60 min at 37 °C. Perchloric acid (35%) was added to the homogenate and centrifuged for 10 min at $40,000 \times g$. The supernatant was mixed with 1.2% thiobarbituric acid, and the mixture was heated at 98 °C for 30 min. After cooling to room temperature, the absorbance was measured at 532 nm. The standard curve was obtained using 1,1,3,3-tetramethoxypropane. The results were expressed as nmol/g of tissue.

Pancreatic non-protein sulfhydryl groups (NP-SH)

The pancreatic NP-SH (reduced glutathione, GSH) were determined by Ellman's reaction using 5'-dithio-bis-2-nitrobenzoic acid (DTNB) (Sedlak and Lindsay, 1968). Aliquots of 4 mL of the homogenates in ice-cold ethylenediaminetetraacetic acid (EDTA; 0.02 mol/L; pH 8.9) were mixed with 3.2 mL of distilled water and 0.8 mL of 50% trichloroacetic acid (TCA). The tubes were centrifuged at $3000 \times g$ for 15 min. The supernatant (2 mL) was mixed with 4 mL Tris buffer (0.4 mol/L; pH 8.9) and 0.1 mL of DTNB (0.01 mol/L). The absorbance was measured within 5 min after addition of DTNB at 412 nm. The absorbance values were extrapolated from a glutathione standard curve. The results were expressed as μ g/g of tissue.

Histological examinations

Samples of pancreatic tissue were fixed in 10% buffered formalin solution, embedded in paraffin using standard methods, cut into 5-mm sections, stained with hematoxylin–eosin, and then assessed under light microscopy and examined blind by a morphologist for

Table 1

Effects of 1,8-cineole and thalidomide on serum amylase and lipase activities and on the pancreatic weight/body weight ratio in mice.

Treatment	Amylase (U/dL)	Lipase (U/L)	Pancreatic weight/body weight ratio (mg/g)
Normal	2985 ± 228.7	710.6 ± 108.8	4.45 ± 0.08
Vehicle	7298 ± 213.5 ^a	1002.0 ± 159.1 ^a	6.78 ± 0.31 ^a
1,8-cineole 100 mg/kg	6276 ± 379.9 ^{ab}	504.7 ± 56.6 ^b	6.34 ± 0.10 ^a
1,8-cineole 200 mg/kg	6076 ± 274.2 ^{ab}	519.6 ± 95.3 ^b	4.90 ± 0.18 ^b
1,8-cineole 400 mg/kg	5768 ± 306.4 ^{ab}	577.2 ± 86.5 ^b	5.60 ± 0.28 ^b
Thalidomide 200 mg/kg	5973 ± 236.5 ^{ab}	599.0 ± 77.4 ^b	5.56 ± 0.21 ^b

Values are expressed as mean ± S.E.M. for 8 animals in each group.

^a $p < 0.05$ compared with the normal group.

^b $p < 0.05$ compared with the vehicle group (ANOVA followed by the Student Newman Keul's test).

grading the histological alterations. Pancreatic edema, leukocyte infiltration, acinar vacuolization, and necrosis were described with scores ranging from 0 to 3 as described by Dembiński et al. (2008).

Immunohistochemistry for NF- κ B/p65

Immunohistochemical analysis of the expression of NF- κ B was performed using EnVision™ FLEX, High pH, kit (Code K8010, Dako, Glostrup, Denmark) according to the manufacturer's instructions. Sections of pancreas (4 μ m) were transferred to silanized slides. Sections were deparaffinized and rehydrated through xylene and graded alcohols. The slides were immersed into preheated (95–99 °C) Target Retrieval Solution (EnVision™ FLEX, Dako, Glostrup, Denmark) in water bath for 30 min. Endogenous peroxidase was blocked with

Peroxidase-Blocking Reagent (EnVision™ FLEX, Dako, Glostrup, Denmark) for 10 min and the nonspecific binding was blocked with goat serum (1:200 in PBS) for 45 min. Slides were incubated with primary rabbit anti-NF κ B p50 subunit (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:200 diluted in Antibody Diluent (EnVision™ FLEX, Dako, Glostrup, Denmark) for 60 min and then incubated with secondary antibody polymer HRP (EnVision™ FLEX, Dako, Glostrup, Denmark) for 30 min. Finally, the sections were stained with 3,3'-diaminobenzidine (DAB) plus chromogen (EnVision™ FLEX, Dako, Glostrup, Denmark) and counterstained with methyl green.

Statistical analysis

The nonparametric data are expressed as median (with low and high ranges), and parametric data as mean ± standard error of the mean (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Kruskal–Wallis or Student–Newman–Keul as post-hoc tests. All data were analyzed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). Statistical differences were considered to be significant at $p < 0.05$.

Results

As shown in Table 1, the secretagogue cerulein (50 mg/kg/h, $\times 6$, i.p.) caused significantly ($p < 0.05$) enhanced levels of serum amylase and lipase when compared to values seen in vehicle-treated controls. Besides, cerulein markedly increased ($p < 0.05$) the pancreatic wet weight/body weight ratio, an index of pancreatic edema. While oral treatment with 1,8-cineole (100, 200 and 400 mg/kg) significantly ($p < 0.05$) lowered the cerulein-induced elevation of serum amylase and lipase at all doses,

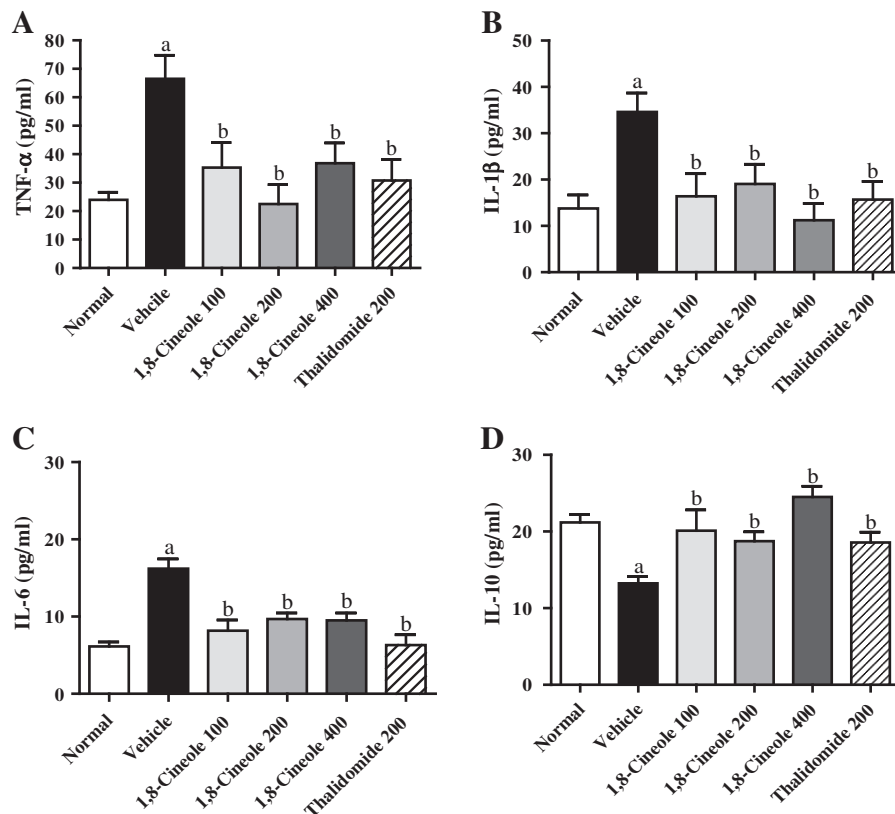


Fig. 1. Effect of 1,8-cineole and thalidomide on serum TNF- α , IL-1 β , IL-6, and IL-10 levels. Mice were pretreated with 1,8-cineole (100, 200, 400 mg/kg, p.o.) or thalidomide (200 mg/kg, p.o.) 1 h before cerulein administration. The vehicle group was treated with 2% Tween 80 in saline. The normal control mice were given saline (0.9% NaCl, i.p.) instead cerulein. Acute pancreatitis was induced by five injections of cerulein (50 μ g/kg, i.p.) at intervals of 1 h. Serum TNF- α (A), IL-1 β (B), IL-6 (C) and IL-10 (D) were determined 6 h after the last cerulein administration. The data are expressed as the mean ± S.E.M. ($n = 8$ animals for group). ^a $p < 0.05$, compared with the normal control and ^b $p < 0.05$, compared with the vehicle control (ANOVA followed by Student Newman Keul's test).

a significant decrease in pancreatic edema was observed only at 200 and 400 mg/kg (Table 1). Lipase inhibition appeared more prominent than to amylase with 1,8-cineole. Thalidomide (200 mg/kg, p.o.) manifested similar reductions in serum amylase and lipase activities, as well as in pancreatic edema.

Cerulein significantly ($p < 0.05$) elevated the serum levels of TNF- α , IL-1 β , and IL-6, whereas the level of serum IL-10 was decreased (Fig. 1). Treatment with 1,8-cineole (100, 200 and 400 mg/kg, p.o.) and thalidomide (200 mg/kg, p.o.) effectively decreased the levels of TNF- α , IL-1 β , and IL-6 but the levels of IL-10 were increased (Fig. 1).

Cerulein caused a significant ($p < 0.05$) increase in pancreatic MPO activity and MDA levels and a decrease ($p < 0.05$) of pancreatic NP-SH (Fig. 2). These changes were reversed by pretreatment with 1,8-cineole (100, 200 and 400 mg/kg, p.o.) and thalidomide (200 mg/kg) in a significant manner.

Histological examination of the normal control showed normal architecture and absence of edema, leukocyte infiltration, acinar vacuolization, hemorrhage and necrosis (Fig. 3A and Table 2). In contrast, pancreatic sections from the cerulein group revealed extensive tissue damage that was characterized by significant disruption of normal architecture, with massive edema, acinar cell vacuolization, necrosis, hemorrhage and inflammatory cell infiltration, and thus received significantly higher scores (Fig. 3B and Table 2). Treatment with 1,8-cineole (200 mg/kg, p.o.) and thalidomide (200 mg/kg, p.o.)

reduced the inflammation, and most strikingly the edema, and protected the pancreas from histological damage induced by cerulein. In addition, the total pathological scores were significantly decreased by 1,8-cineole and thalidomide treatment (Fig. 3C and d and Table 2).

Representative NF- κ B immunostaining of the pancreas for different treatments is shown in Fig. 4. In normal control, the pattern of NF- κ B staining was very mild (Fig. 4A). On the other hand, there was a high intensity staining for NF- κ B in the acinar cells, inflammatory cells and blood vessels of the pancreas in the cerulein group (Fig. 4B), however, in mice treated with 1,8-cineole (200 mg/kg, p.o.) or thalidomide (200 mg/kg, p.o.), immunostaining intensity for NF- κ B was much less (Fig. 4C and D).

Discussion

Studies reveal that in acute pancreatitis (AP), pancreatic acinar and stellate cells play an important role in leukocyte attraction via secretion of chemokines, cytokines and expression of adhesion molecules (Gukovskaya et al., 1997; Mews et al., 2002; Vonlaufen et al., 2008). Neutrophils in particular exert their toxicity by releasing myeloperoxidase (MPO), proteases such as elastase and reactive oxygen species (ROS) such as H₂O₂. Cerulein is an analog of cholecystikinin (CCK) and by its interaction with pancreatic acinar cells causes the maximum pancreatic secretion of amylase and lipase (Jensen et al., 1989), cytoplasmic vacuolization, death of acinar cells, edema formation, and infiltration of inflammatory cells into the pancreas (Gorelick et al., 1993), simulating the characteristics of human pancreatitis. The mechanism of action of cerulein is suggested to involve production of large amounts of ROS, activation of oxidant-sensitive transcription factor nuclear factor- κ B (NF- κ B) and induction of cytokine expression in pancreas (Yu and Kim, 2012).

In our study, very high levels of MPO activity and MDA with a low level of reduced glutathione were observed in cerulein-induced AP, indicating that cerulein induces an oxidative stress, a finding consistent with an earlier report (Jensen et al., 1989). The observed decreases in the levels of MPO and MDA with an elevated glutathione in 1,8-cineole-pretreated mice suggests that it protects the pancreatic tissues from oxidative damage induced by cerulein, and this effect possibly involves the inhibition of neutrophil infiltration and lipid peroxidation.

In the diagnosis of AP, serum amylase and lipase remain important tests with a high specificity. However, the major advantage of lipase is an increased sensitivity in acute pancreatitis and in patients it remains elevated longer than amylase (Yadav et al., 2002). Studies even consider that serum pancreatic lipase is a more accurate biomarker of acute pancreatitis than serum amylase (Smith et al., 2005). In this study, 1,8-cineole although effectively reduced the cerulein-induced increase in both lipase and amylase levels, cineole effect appeared more pronounced on lipase, showing its relevance to human AP.

In addition to enhanced serum amylase and lipase activities, this study noticed very high levels of proinflammatory cytokines, TNF- α , IL-1 β , and IL-6 and a low level of anti-inflammatory cytokine IL-10 in the serum of cerulein-induced AP. Activated acinar cells and migrating cells release these pro-inflammatory cytokines in response to the local damage in the pancreas (Kusske et al., 1996). One of the early events in the development of AP is the release of these cytokines from pancreatic tissue, and the level of cytokine release is related to the degree of pancreatic inflammation (Mayer et al., 2000), and their blockade attenuates the disease process. In our experiment, these changes in cytokine levels were significantly mitigated by 1,8-cineole pretreatment, suggesting that it may have an important role in the inhibition of the pro-inflammatory response in AP. By creating a balance between pro-inflammatory and anti-inflammatory cytokines, 1,8-cineole might exercise a therapeutic benefit for the control of acute pancreatitis. In this regard, previous studies provided evidence for the role of 1,8-cineole in the control of airway mucus

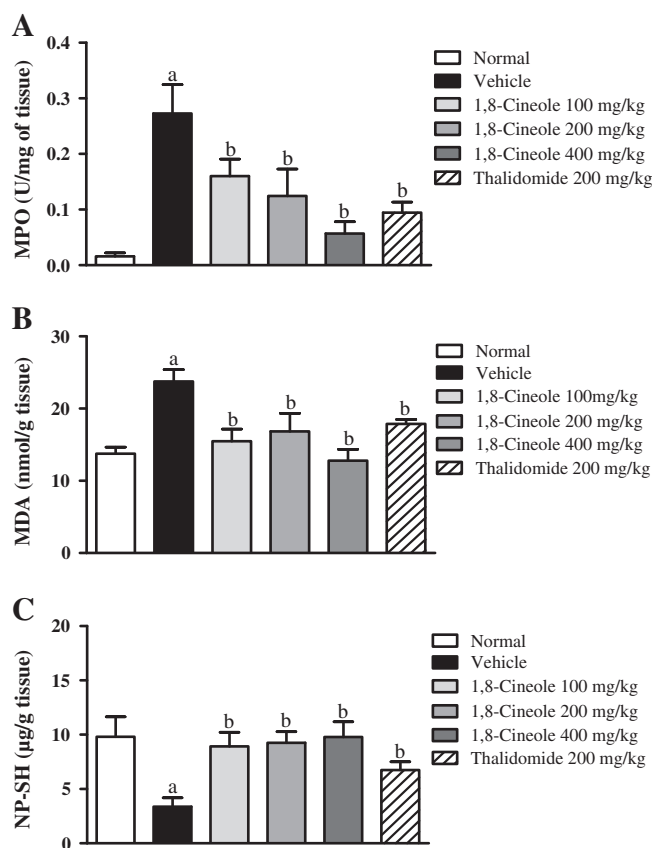


Fig. 2. Effect of 1,8-cineole and thalidomide on pancreatic MPO, MDA and NP-SH. Mice were pretreated with 1,8-cineole (100, 200, 400 mg/kg, p.o.) or thalidomide (200 mg/kg, p.o.) 1 h before cerulein administration. The vehicle group was treated with 2% Tween 80 in saline. The normal control mice were given saline (0.9% NaCl, i.p.) instead of cerulein. Acute pancreatitis was induced by five injections of cerulein (50 μ g/kg, i.p.) at intervals of 1 h. Pancreatic myeloperoxidase (MPO) (A), malondialdehyde (MDA) (B) and non-protein sulfhydryls (NP-SH) (C) were determined 6 h after the last cerulein administration. The data are expressed as the mean \pm S.E.M. ($n = 8$ animals for group). ^a $p < 0.05$, compared with the normal control and ^b $p < 0.05$, compared with the vehicle control (ANOVA followed by Student Newman Keul's test).

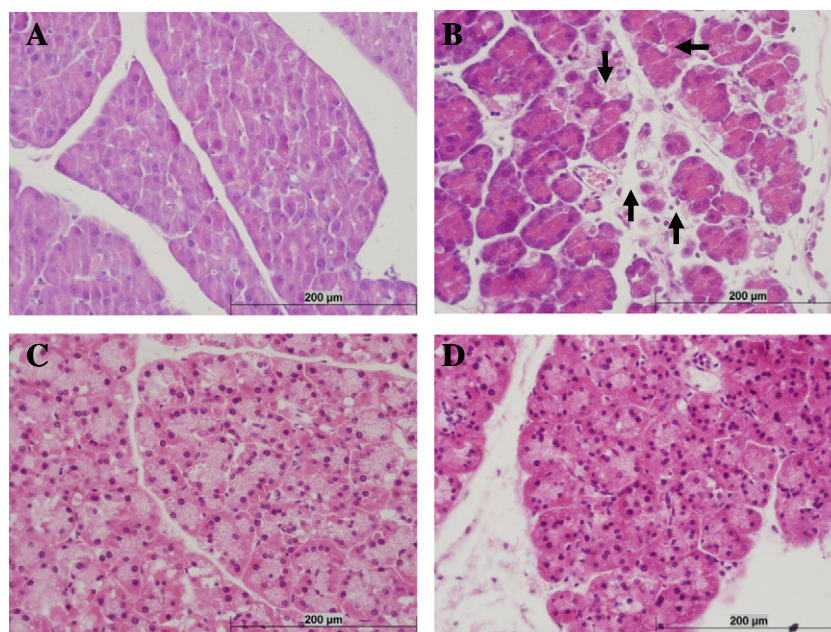


Fig. 3. Representative photomicrographs of the pancreas. Mice were pretreated with 1,8-cineole (200 mg/kg, p.o.) or thalidomide (200 mg/kg, p.o.) 1 h before cerulein administration. The vehicle group was treated with 2% Tween 80 in saline. The normal control mice were given saline (0.9% NaCl, i.p.) instead cerulein. Acute pancreatitis was induced by five injections of cerulein (50 µg/kg, i.p.) at intervals of 1 h. The pancreas was examined histologically 6 h after the last cerulein administration. Normal control group showing normal architecture (A), pancreas of animals treated with vehicle + cerulein showing disruption of pancreatic architecture with acinar cell vacuolization, acinar cell necrosis, and inflammatory cell infiltration (B), 1,8-cineole (200 mg/kg) + cerulein (C), and thalidomide (200 mg/kg) + cerulein (D) showing reduction of acinar cell vacuolization, acinar cell necrosis and inflammatory cell infiltration. Hematoxylin and eosin ($\times 400$).

hypersecretion by cytokine inhibition, suggesting that long-term treatment with 1,8-cineole can reduce exacerbations in asthma, sinusitis and chronic obstructive pulmonary disease (Juergens et al., 2003, 2004).

NF- κ B is a nuclear transcription factor responsible for regulating the transcription of a wide variety of genes involved in immunity and inflammation. This factor has been shown to play a critical role in the development of acute pancreatitis (Rakonczay et al., 2008). In addition, pro-inflammatory cytokines, particularly TNF- α , and oxidative stress promote each other generating a vicious circle in acute pancreatitis. Mice given injections of cerulein, to induce acute pancreatitis, had higher levels of NF- κ B activity in pancreatic acinar cells than control mice, consistent with earlier observations that correlate the level of NF- κ B activation with the severity of acute pancreatitis in mice (Huang et al., 2013). Given the importance of TNF- α and NF- κ B in the pathogenesis of AP (Malleo et al., 2008; Pongratz et al., 2013), blocking the action of these mediators by 1,8-cineole may be an attractive treatment option to treat patients with AP.

Acute pancreatitis remains the most frequent complication of endoscopic retrograde cholangiopancreatography (ERCP) for which an ideal prophylactic drug is still not available. However, recently, a randomized prospective controlled trial of rectal indomethacin revealed

significant reduction in the incidence of ERCP pancreatitis (Cheon, 2013). It remains to be seen if rectal administration of 1,8-cineole might also be a useful prophylactic measure to prevent ERCP associated pancreatitis without collateral effects.

Although 1,8-cineole (200 mg/kg), reduced cerulein-induced increase of amylase only to a less extent, histological evidence projects its marked protection against AP. The mild reduction of amylase by 1,8-cineole compared to lipase might be the chemical characteristic of this particular monoterpene. Another observation is that cineole does not manifest dose-related responses in many of the observed parameters. The responses were measured in relation to increasing doses, expressed in terms of mg/kg body weight and not in relation to blood level concentrations (µmol/L) of 1,8-cineole. Past studies indicate that it has low bioavailability following the oral route, the reasons being its rapid absorption, larger volume of distribution, storage in fat tissues, and a rapid metabolism by liver cytochrome P450 enzymes (CYP3A4). Another reason might be that the plasma concentrations may not elevate with the increased doses due to simultaneously enhanced metabolism of cineole by liver cytochrome P450 enzymes (as it occurs in brushtail possum—*Trichosurus vulpecula*) (McLean et al., 2007), resulting in increased blood levels of 2-hydroxy-cineole (major metabolite of 1,8-cineole) but not the parent compound 1,8-cineole (Miyazawa et al., 2001). However, this notion requires a bioavailability study analyzing the blood level concentrations of cineole in relation to different doses given orally.

1,8-cineole (200 mg/kg) reduced the pancreatic weight (PW)/body weight (BW) ratios as well as the TNF- α almost to the levels seen in normal controls, which signify its histological evidence of protection against cerulein-induced pancreatic damage. Since 1,8-cineole could effectively modulate the NF- κ B activation associated inflammatory response, we assume that its anti-inflammatory activity might play a major role in the amelioration of cerulein-induced acute pancreatitis.

Pain management in pancreatic disease remains a major clinical concern. The transient receptor potential ankyrin 1 (TRPA1) channel is well known to be affected by oxidative stress as shown in the experimental models of itch or neuropathic pain (Liu and Ji, 2012;

Table 2

Effects of 1,8-cineole and thalidomide treatment on morphological signs of pancreatic damage.

Treatment	Edema (0–3)	Inflammatory infiltration (0–3)	Acinar vacuolization (0–3)	Necrosis (0–3)
Normal	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Vehicle	2.87 (2–3) ^a	2.75 (2–3) ^a	2.87 (2–3) ^a	2.62 (2–3) ^a
1,8-cineole 200 mg/kg	0.25 (0–1) ^b	0.25 (0–1) ^b	0.75 (0–1) ^b	0.37 (0–1) ^b
Thalidomide 200 mg/kg	0.50 (0–1) ^b	0.63 (0–1) ^b	0.87 (0–2) ^b	0.62 (0–1) ^b

Values are expressed as median scores ranges (min–max) for 8 animals in each group.

^a $p < 0.05$ compared with the normal group.

^b $p < 0.05$ compared with the vehicle group (ANOVA followed by the Kruskal–Wallis test).

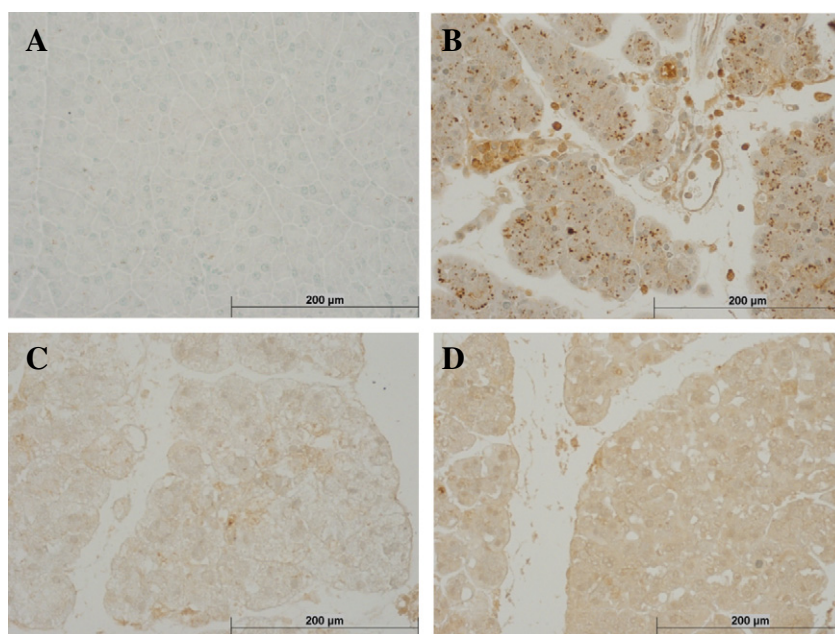


Fig. 4. Effects of 1,8-cineole and thalidomide on NF- κ B immunoreactivity in cerulein-induced acute pancreatitis. Mice were pretreated with 1,8-cineole (200 mg/kg, p.o.) or thalidomide (200 mg/kg, p.o.) 1 h before cerulein. The vehicle group was treated with 2% Tween 80 in saline. The normal control mice were given intraperitoneal saline (0.9% NaCl) solution instead of cerulein. Acute pancreatitis was induced by five intraperitoneal injections of cerulein (50 μ g/kg) at intervals of 1 h. NF- κ B expression was measured by immunohistochemistry 6 h after the last dose of cerulein. Normal control group showing basal immunostaining (A), pancreas of animals treated with vehicle showing disruption of pancreatic architecture with acinar cell vacuolization, acinar cell necrosis, and inflammatory cell infiltration (B), 1,8-cineole (200 mg/kg, p.o.) (C), and thalidomide (200 mg/kg, p.o.) (D) showing immunostaining comparable to the control group.

Materazzi et al., 2012), and so is similarly in the pathogenesis of pancreatitis and the associated inflammation and pain that could be attenuated by TRPA1-channel antagonists (Schwartz et al., 2011). Since 1,8-cineole has recently been reported to function as a TRPA1 inhibitor (Takaishi et al., 2012), it may be considered as an ideal molecule for drug development, to prevent or treat patients with acute pancreatitis.

Conclusion

In conclusion, this experimental study reveals that 1,8-cineole by preventing the oxidative stress, inflammation, and acinar cell injury attenuates the development of cerulein-induced acute pancreatitis in mice. 1,8-cineole may be in particular useful as prophylaxis against therapeutic drugs-induced and endoscopic retrograde cholangiopancreatography (ERCP)-associated pancreatitis. However, further detailed experimental and clinical evaluation on this monoterpenoid is necessary in the development of new therapeutic strategies for treatment of acute pancreatitis.

Conflict of interest statement

The authors declare that they have no competing interests.

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