

REVIEW

Pharmacology and preclinical pharmacokinetics of peppermint oil

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Abstract

The principal pharmacodynamic effect of peppermint oil relevant to the gastrointestinal tract is a dose-related antispasmodic effect on the smooth musculature due to the interference of menthol with the movement of calcium across the cell membrane. The choleric and antifoaming effects of peppermint oil may play an additional role in medicinal use.

Peppermint oil is relatively rapidly absorbed after oral administration and eliminated mainly via the bile. The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation. The urinary metabolites result from hydroxylation at the C-7 methyl group at C-8 and C-9 of the isopropyl moiety, forming a series of mono- and dihydroxymenthols and carboxylic acids, some of which are excreted in part as glucuronic acid conjugates. Studies with tritiated I-menthol in rats indicated about equal excretion in feces and urine. The main metabolite identified was menthol-glucuronide. Additional metabolites are mono- or di-hydroxylated menthol derivatives.

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Keywords: Peppermint oil; Spasmolysis; Calcium antagonist; Antifoaming activity; Choleresis; Pharmacokinetics**Introduction**

Peppermint oil has a long history of safe use both in medicinal preparations and as a flavoring agent in foods and confectionery. Peppermint oil is indicated for both external and internal use. In an ESCOP monograph in 1997 (ESCOP, 1997) the medicinal use is summarized and FDA granted the oil a so-called “generally recognized as safe” (GRAS) status (Food and drugs, 1998).

The major constituents of the oil include the terpenes (–)-menthol (30–55%), (–)-menthone (14–32%), (+)-isomenthone (1.5–10%), (–)-menthyl acetate (2.8–

10%), (+)-menthofuran (1.0–9.0%) and 1,8-cineol (3.5–14%).

The purpose of this review is to summarize preclinical research data with peppermint oil or its constituents, relevant to the gastrointestinal tract.

Dynamics and mode of action

The antispasmodic activity of 2.5 and 10.0 ml/l of alcoholic extracts of *Melissa officinalis*, *Rosmarinus officinalis*, *Mentha piperita*, *Matricaria chamomilla*, *Foeniculum vulgare*, *Carum carvi* and *Citrus aurantium* prepared from 1 part of the plant and 3.5 parts of ethanol (31% w/w) was tested employing the guinea pig ileum and using acetylcholine and histamine as spasmogens (Forster et al., 1980). Most of the extracts

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shifted the dose response curves of acetylcholine and histamine to the right in a dose-dependent manner. Extracts from *Carum carvi*, *Mentha piperita*, *Citrus aurantium* and *Matricaria chamomilla* showed a significant rise of the DE_{50} of acetylcholine-induced contractions and a significant decrease of the maximal possible contractility. In histamine-induced contractions, all plant extracts except Extractum Melissae exhibited a significant increase of the DE_{50} , and all extracts used decreased the maximal possible contractility produced by histamine. The alcoholic extract of *Mentha piperita* was most effective when tested with acetylcholine and the extract of *Citrus aurantium* was most active when tested with histamine. *Melissa officinalis* did not show significant antispasmodic activity. When the antispasmodic activities of the most effective plant extracts were compared with the activity of atropine, it was evident that their effects were less than that of the usual therapeutic dosage of atropine in man. The most pronounced effects with 10 ml/l Extractum Citrus aurantii and 10 ml/l Extractum Menthae piperitae correspond to the effect of 0.07 resp. 0.13 mg atropine.

The antispasmodic effects of peppermint oil have been directly demonstrated *in vitro* in a series of experiments with smooth muscle fibres isolated from the guinea pig, including the trachea and ileum (Reiter and Brandt, 1985; Taddei et al., 1988) and the sphincter of Oddi (Giachetti et al., 1988). Peppermint oil was shown to be effective in reducing muscle tone in all three systems either at rest or following electrical stimulation or morphine treatment. The effects of peppermint oil were shown to be due largely to its menthol constituent.

Evans et al. (1975) studied the effect of menthol on the colonic motility in dogs. Biological activity was estimated on colonic motility using mongrel dogs with an exteriorized terminal ileum which allowed direct access to the colon and which had been kept in continuity with both the ileum and the remainder of the large intestine. Each dog was given an enema on the day prior to experimentation and colonic motility was examined by recording intra-luminal pressure with three water-filled polyethylene tubes inserted through the ileal stoma into the proximal, medial and distal regions and linked to a multi-channel pen-recorder. A recording of the normal pattern of colonic motility was first obtained following the introduction of 30 ml normal saline into the colon. A similar volume of menthol, at a concentration of 1.0 mg/ml, was then introduced into the colon and the effect on intra-luminal pressure recorded. It produced an immediate decrease in colonic motility, which lasted from 20–25 min, before the normal pattern of motility was restored. Decreased motility was observed as a reduction in intra-luminal pressure.

Hills and Aaronson (1991) studied the mechanism of action of peppermint oil on gastrointestinal smooth muscle in isolated organs. Peppermint oil relaxed

carbachol-contracted guinea pig taenia coli (IC_{50} 22.1 μ g/ml) and inhibited spontaneous activity in the guinea pig colon (IC_{50} 25.9 μ g/ml) and rabbit jejunum (IC_{50} 15.2 μ g/ml). Peppermint oil markedly attenuated contractile responses in the guinea pig taenia coli to acetylcholine, histamine, 5-hydroxytryptamine, and substance P. Peppermint oil reduced contractions evoked by potassium depolarization and calcium contractions evoked in depolarizing Krebs solutions in taenia coli. Potential-dependent calcium currents recorded using the whole cell clamp configuration in rabbit jejunum smooth muscle cells were inhibited by peppermint oil in a concentration-dependent manner. Peppermint oil both reduced peak current amplitude and increased the rate of current decay. The effect of peppermint oil resembled that of dihydropyridine calcium antagonists. The authors concluded that peppermint oil relaxes gastrointestinal smooth muscle by reducing calcium influx.

Taylor et al. (1985) studied in guinea pig isolated ileum and human isolated taenia coli the relaxant activity of peppermint oil and its constituents. Carbachol was used as antagonist. Doses required to bring about 50% relaxation (ID_{50}) were then compared. Menthol (3.0×10^{-5} w/w) was the most active constituent, being more active than peppermint oil (4.4×10^{-5} w/w) while menthone, menthyl acetate and cineole were considerable less active than peppermint oil. Using strips (30×3 mm) of human isolated taenia coli suspended in normal Krebs' solution bubbled with 5% CO_2 in O_2 at 37 °C, peppermint oil and menthol inhibited basal tone and contractions to carbachol (10^{-7} – 10^{-4} M) and to potassium chloride (5–150 mM) in a non-competitive manner. In calcium-free, depolarizing Krebs' solution (mM: NaCl 82.7; KCl 40.0; $NaHCO_3$ 25.0; NaH_2PO_4 1.4; glucose 11.5) parallel shifts in dose response curves to calcium (0.1–20 mM) indicated that peppermint oil and menthol possess specific calcium antagonist activity. To further investigate this effect the influx of $^{25}Ca^{2+}$ (μ mol/kg wet weight) into carbachol (10^{-6} M) or potassium (80 mM) stimulated rings of guinea pig ileum (7–15 mg) suspended in buffered HEPES solution containing $^{45}Ca^{2+}$ (10^{-5} = Ci/ml) was studied. Following carbachol or potassium stimulation the extracellular concentration of Ca^{2+} increased significantly ($p < 0.001$). However, in the presence of menthol (0.64 = M) no such influx was observed. The calcium antagonist, verapamil (10^{-5} M) likewise inhibited $^{45}Ca^{2+}$ uptake in response to carbachol and potassium stimulation. In addition, peppermint oil and menthol inhibited carbachol-induced contractions of the guinea pig isolated ileum suspended in calcium-free Tyrode's solution in the readmission of calcium ions, further indicating that peppermint oil and menthol are able to inhibit carbachol-induced influx of extracellular calcium ions.

The effect of peppermint oil and menthol on isolated human coli was investigated by Taylor et al. (1984). A total of 50 strips of taenia, approximately 30×3 mm, were dissected from 20 resections for carcinoma. Peppermint oil and menthol produced both inhibition of spontaneous activity and decrease in basal tone in all tissues in a dose-dependent manner. Under isotonic conditions (tension 2 g), ID_{50} values (concentration of antagonist producing 50% reduction in response to carbachol, 10^{-6} M) were calculated for menthol (0.29 ± 0.11 mM; $n = 5$) and for peppermint oil (0.41 ± 0.06 mM; $n = 5$; estimated MW 160). Under isometric conditions, dose–response curves to carbachol (10^{-7} – 10^{-4} M and to potassium (5–150 mM) demonstrated non-competitive inhibition by both peppermint oil and menthol, this effect being rapidly reversible on wash-out. In calcium-free, depolarizing Krebs's solution (mM; NaCl 82.7; KCl 4.0; $NaHCO_3$ 25.0; NaH_2PO_4 1.4; glucose 11.5), dose–response curves to calcium (0.1–20 mM) showed a specific calcium antagonist effect of menthol, which was dose-related and rapidly reversible.

Beesley et al. (1996) studied the influence of peppermint oil on absorptive and secretory processes in rat small intestine using both intestinal sheets mounted in Ussing chambers and brush border membrane vesicles. Peppermint oil in the intestinal lumen inhibited enterocyte glucose uptake via a direct action on the brush border membrane. Intestinal secretion was inhibited by peppermint oil, which is consistent with a reduced availability of calcium.

The action of menthol and/or peppermint oil as a calcium channel antagonist has been demonstrated *in vitro* by Hawthorn et al. (1988). These investigators used $^{45}Ca^{2+}$ uptake and radioligand-binding assays to measure the effects of menthol and peppermint oil in a range of mammalian tissues. Both showed Ca^{2+} channel-blocking activity in guinea-pig ileum, rat and guinea-pig cardiac muscle, rat brain synaptosomes and also in chick retinal neurones. The results of binding studies supported a Ca^{2+} channel-specific effect in both cases.

Palade et al. (1989) classified menthol as activator of the Ca^{2+} -induced Ca^{2+} release in sarcoplasmic reticulum, which more than doubled the control rate of ruthenium red-insensitive unidirectional ^{45}Ca efflux. Rampe and Trigg (1990) reviewed new ligands for L-type Ca^{2+} channels of different chemical structure, among them menthol. The identity of the binding site has not yet been established; however, such ligands were proposed for new directions of Ca^{2+} channel drug structures. Zygmunt et al. (1993) performed structure activity studies on the calcium antagonistic properties of terpenes and suggested that these substances represent a new chemical class of calcium antagonists, which interact with dihydropyridine binding sites.

Using isolated ganglia from *Helix pomatia* and cultured dorsal root ganglion cells from chick and rat embryos, Swandulla et al. (1986, 1987), Schäfer et al. (1988) found that menthol blocks currents through the low-voltage-activated Ca channel, and facilitates inactivation gating of the classical high voltage-activated Ca channel. Schäfer et al. (1995) reported similar findings in afferent discharges from electroreceptor organs of catfish.

These results indicate that the spasmolytic effect of peppermint oil on the intestinal smooth musculature appears to involve calcium antagonism. Menthol, which is the major constituent of peppermint oil exerts its effect most probably via a calcium channel antagonistic effect. This leads to antispasmodic activity observed in pharmacodynamic studies with peppermint oil or menthol. The activity appears to be dose dependent. The mechanism by which this is brought about is associated with the ability of menthol to decrease the influx of extracellular calcium ions through potential dependent channels.

Antifoaming activity

Harries et al. (1978) studied in an apparatus specially designed to assess foams in digestive fluids *in vitro* the antifoaming effect of various carminatives. The effects of caraway, cinnamon, dill, orange and peppermint oils on gastric and intestinal foams were examined. Reductions in foam volume were observed in every case, although the effects were not as great as those produced by a combination of dimethicone and silica. *m*-Cresol, *p*-hydroxybenzaldehyde, isobutanol, menthol and phenoxethanol also reduced foam volume. It is suggested that carminative action is a combination of effects, one of which is a reduction of gastrointestinal foam.

Choleretic activity

A choleretic action has traditionally been ascribed to peppermint oil, in keeping with the occasional use of menthol in the treatment of gallstones (Leuschner et al., 1988). Increases in bile production of 1.3–2.4-fold were recorded (Mans and Pentz, 1987) after oral doses of 0.1–1.0 g/kg bw in male rats. Trabace et al. (1993) found dose- and time-dependent choleretic effects of peppermint oil and menthol in anesthetized rats and used these as standards for testing other essential oils. The mechanism of action underlying the observed choleretic activity of menthol or other constituents of peppermint oil is not clearly understood, but may result from the marked biliary output of metabolized menthol (Mans and Pentz, 1987).

Pharmacokinetics

Mans and Pentz (1987) studied the pharmacokinetic behaviour of menthol administered orally at a range of doses (0.1–1.0 g/kg b.w.) in male rats. Plasma levels and biliary and renal excretion of unchanged versus conjugated menthol were measured. The levels of unchanged compound were low in plasma, bile and urine, with a preponderance of the glucuronide (60%) present in the urine and of the sulphate (60–90%) present in the bile. Renal recovery of total menthol within 24 h was dose dependent, ranging from 5.4% (0.1 g/kg) to 2.1% (1 g/kg). The recovery in bile over 8 h was substantially higher, ranging from 16% to 6% at the corresponding dose levels. Dose-related increases in volume of both urine and bile were recorded.

Biotransformation

Madyastha and Srivatsan (1988) investigated the metabolism of *l*-menthol in rats both *in vivo* and *in vitro*. Metabolites isolated and characterized from the urine of rats after oral administration (800 mg/kg) of *l*-menthol were the following: *p*-menthane-3,8-diol, *p*-menthane-3,9-diol, 3,8-oxy-*p*-menthane-7-carboxylic acid, and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. Repeated administration of 800 mg/kg *l*-menthol to rats for 3 days resulted in the increase of both liver mitochondrial cytochrome P-450 content and NADPH-cytochrome C reductase activity by nearly 80%. Rat liver microsomes readily converted *l*-menthol to *p*-menthane-3,8-diol in the presence of NADPH and O₂. A metabolic pathway of *l*-menthol in rats was proposed. Yamaguchi et al. (1994) administered [3-³H]-*l*-menthol by oral gavage to intact and bile duct-cannulated male Fischer 344 rats at a dose level of 500 mg/kg. Excreta were collected for up to 48 h and metabolites in urine and bile analysed by TLC, solid phase extraction, GLC, and GC/MS. In intact rats, some 71% of the dose was recovered in 48 h with approximately equal amounts in urine and feces. In total, 74% of the dose was recovered from bile duct-cannulated rats, with 67% in the bile and 7% in the urine. The major biliary metabolite was menthol glucuronide, which undergoes enterohepatic circulation. The urinary metabolites resulted from hydroxylation at the C-7 methyl group at C-8 and C-9 of the isopropyl moiety, resulting in a series of mono- and dihydroxymenthols and carboxylic acids, some of which are excreted in part as glucuronic acid conjugates. The results enabled the construction of a metabolic map for menthol in the rat.

A twofold difference in the formation rate of glucuronides of (+)- and (–)-menthol by rat liver slices and by rat liver microsomes was found by Caldwell

(1995). The plasma elimination half-life of (–)-menthol is 2.4 h compared with 4.0 h for (+)-menthol, with the plasma AUC of (–)-menthol being threefold less than for the (+)-isomer. These pharmacokinetic differences arise from the enormous difference between the isomers in terms of the biliary excretion of their glucuronides: 69% of a dose of the more rapidly cleared (–)-menthol is excreted in the bile in 24 h compared with only 32% for (+)-menthol.

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