A Novel Ileocolonic Release Peppermint Oil Capsule for Treatment of Irritable Bowel Syndrome

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ORIGINAL RESEARCH

A Novel Ileocolonic Release Peppermint Oil Capsule for Treatment of Irritable Bowel Syndrome: A Phase I Study in Healthy Volunteers

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ABSTRACT

Introduction: Peppermint oil (PO) has been shown to reduce abdominal pain in patients with irritable bowel syndrome (IBS). PO is assumed to induce intestinal smooth muscle relaxation and desensitization of nociceptive nerve afferents. To increase colonic PO concentration, an ileocolonic release peppermint oil (IC-PO) capsule has been developed. The aim of this study was to compare pharmacokinetic parameters of the currently available small intestinal release PO (SI-PO) and the novel IC-PO.

Methods: In this randomized, double-blind, crossover study, subjects received 182 mg of either SI-PO or IC-PO in a crossover design with a washout period of more than 14 days. Blood samples were collected to determine menthol glucuronide concentrations.

Results: Eight healthy volunteers (50% female, median age 22) were included. The time to reach the maximum concentration (T_{max}) of IC-PO was significantly longer compared to SI-PO with a median (IQR) of 360 (360–405) versus 180 (120–180) min. The lag time (T_{lag}) was significantly longer with a median (IQR) of 225 (204–284) for IC-PO compared to 37 (6–65) min for SI-PO. The areas under the menthol glucuronide plasma concentration–time curves were significantly smaller with a median (IQR) of 2331 μg h/L (2006–2510) for IC-PO compared to 2623 μg h/L (2471–2920) for SI-PO. No

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significant differences were found in peak concentrations and elimination half-lives.
Conclusion: IC-PO has a significantly delayed peak menthol glucuronide concentration and T_{lag}, both pointing to the release of PO in the more distal part of the intestine. This may enhance therapeutic efficacy as it results in increased exposure of colonic mucosal afferents to the PO. A randomized controlled trial investigating the efficacy of SI and IC-PO in IBS is currently ongoing.
Trial registration: ClinicalTrials.gov identifier, NCT02291445, EudraCT database 2014-004195-32.

Keywords: Abdominal pain; Irritable bowel syndrome; Gastroenterology; Peppermint oil; Pharmacokinetics; Phase I; Targeted therapeutics

INTRODUCTION

Irritable bowel syndrome (IBS) is a complex functional bowel disorder affecting up to 10–20% of the population in developed countries [1, 2]. It is characterized by recurrent abdominal pain associated with changes in bowel habits [1]. Of the currently available treatment entities, peppermint oil released in the upper small intestine has been shown to be effective in reducing complaints of abdominal pain and inducing global symptom improvement [3, 4] with a reported number needed to treat (NNT) of 2–3 [5, 6].

The main constituent of peppermint oil is L-menthol, which is rapidly metabolized to menthol glucuronide and excreted in urine when taken orally. The exact mechanism of how peppermint oil acts remains to be elucidated, but is most likely multifactorial [7]. What is known, however, is its dose-related relaxational effect of intestinal smooth muscle through the inhibition of calcium influx into the sarcolemma of smooth muscle cells [8–10] and thereby potentially decreasing abdominal cramps. Furthermore, there are indications that peppermint oil has a direct local antinociceptive effect in the colon through an interaction of L-menthol with transient receptor potential (TRPM8 and/or TRPA1) channels, channels known to play a role in visceral hypersensitivity and pain generation [11–14]. Other studies have reported inhibition of serotonin type 3 receptors (5-HT_3) in the human colon [15], antimicrobial [16, 17], and carminative effects [18]. Capsules containing peppermint oil are available as an over the counter drug on the European market [19] and capsules containing peppermint oil microspheres are available as a medical food product in the USA and Canada [20]. All these formulations release peppermint oil in the small intestine.

The use of small intestinal release peppermint oil is associated with upper gastrointestinal side effects, such as an altered sensation in the mouth in up to 11% and dyspeptic symptoms including heartburn, reflux and belching in up to 24% of patients [21–24]. These burdensome symptoms negatively affect therapy adherence. To decrease these side effects, it could be argued that an ileocolonic release of peppermint oil is beneficial. In addition, a colonic release may increase efficacy in IBS patients by enhancing local colonic relaxation and TRP stimulation. Therefore, a new peppermint oil soft capsule formulation with a predominant distal ileocolonic release has been developed using a previously described ileocolonic delivery technology to coat existing peppermint oil capsules [25–27].

This study aimed to determine the pharmacokinetic differences and safety of both the small intestinal release peppermint oil and the ileocolonic release peppermint oil in an in vitro model and in healthy volunteers. Because the ileocolonic release formulation will release the peppermint oil in the ileocolonic region as opposed to the upper small intestine, we hypothesized that this will result in a delayed and possibly lower peak menthol glucuronide concentration in the plasma compared to plasma concentrations found after administration of small intestinal release capsules.

METHODS

In Vitro GISS Experiment

The novel ileocolonic release capsules were tested in the gastrointestinal simulation system

Table 1 Specifications of the four phases of the dissolution test (GISS)

Phase	Gastrointestinal segment	Volume (mL)	Residence time (h)	pH	Osmolality (modmol/kg)
I	Stomach	500	2.0	1.2 ± 0.10	150 ± 25
II	Jejunum	629	2.0	6.8 ± 0.20	250 ± 50
III	Ileum (distal)	940	0.5	7.5 ± 0.25	250 ± 50
IV	Colon (proximal)	1000	1.5	6.0 ± 0.25	250 ± 60

GISS gastrointestinal simulation system

Adapted from Schellekens et al. [28]. Adapted from *European Journal of Pharmaceutical Sciences*, Vol. 30, Issue 1, R.C.A. Schellekens, F.E. Stuurman, F.H.J. van der Weert, J.G.W. Kosterink, H.W. Frijlink, A novel dissolution method relevant to intestinal release behaviour and its application in the evaluation of modified release mesalazine products, Pages No. 15–20, Copyright (2007), with permission from Elsevier

(GISS). The GISS is an in vitro model based on the pharmacopoeial dissolution test and has been described in detail elsewhere [28]. In summary, the model simulates pH and transit times through the human gastrointestinal tract and enables variation of these and other parameters such as osmolality and agitation [28, 29]. Table 1 shows the four GISS test phases.

Phase I Human Trial

The research protocol was approved by the Maastricht University Medical Center Committee of Ethics and all study procedures were performed in compliance with Good Clinical Practice Guidelines and according to the revised Declaration of Helsinki. The study has been registered in the US National Library of Medicine (NCT02291445) and the EudraCT database (2014-004195-32) and all subjects gave a written informed consent prior to participation.

Subjects

All subjects were healthy, non-smoking volunteers between 18 and 65 years old, with a BMI between 18 and 25 kg/m^2 and no history of gastrointestinal or other chronic disease (as assessed by screening in which medical history, physical examination, and vital signs were checked). Participants were recruited via local advertisements and a national recruitment website. Exclusion criteria included high intake of alcohol (more than 15 consumptions per week) or caffeine (more than eight cups coffee a day). Women of fertile age underwent a standard pregnancy test and were instructed to continue their contraception throughout the study.

Medication use (except for contraception) was prohibited in the 14 days prior to the test period and volunteers were instructed to abstain from alcohol, caffeine, grapefruit, and products containing menthol or peppermint oil such as toothpaste, candy, and mouthwash for 48 h before each test day. Menthol-free toothpaste was provided beforehand.

Study Design

This study is designed according to US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines for bioequivalence studies [30, 31]; a randomized, double blind, two-treatment, single-dose, crossover pharmacokinetic study with a washout period of at least 14, but no longer than 21 days (Fig. 1).

The study consisted of two identical test periods of 24 h in which all participants received 182 mg of ileocolonic and small intestinal release peppermint oil capsules. In line with the European Pharmacopoeia entry for peppermint oil [32], both 182 mg formulations contained between 51 mg and 105 mg of L-menthol (normal variance between capsules, no differences between small intestinal versus ileocolonic release). As the estimated half-life of menthol glucuronide in both preparations was

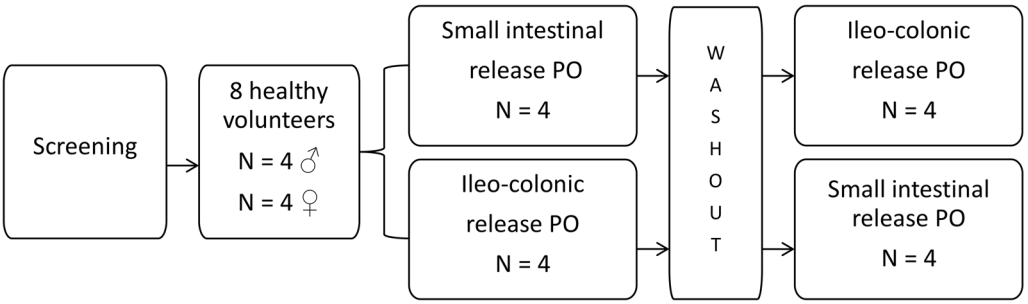

Fig. 1 Study design; after screening, participants (healthy volunteers) were randomized and received small intestinal release peppermint oil (PO) or ileocolonic release PO in a crossover design with a washout period of at most 21 days, but at least 14 days

between 3 and 10 h [25, 33], no carry-over effects were anticipated as a result of the chosen washout period.

Randomization, preparation, and labeling of the study medication were performed by an independent third party (Tiofarma BV, Oud Beijerland, the Netherlands). Half of the subjects received ileocolonic release capsules in the first test period (and small intestinal release capsules in the second test period) and half of the subjects received small intestinal release capsules in the first test period (and ileocolonic release capsules in the second test period) on the basis of a randomized preselection using web-based randomization software. All capsules were packaged in identical, sealed containers and subject numbers and whether it was the first or second test day were mentioned on the label, ensuring allocation concealment.

On both test days, subjects arrived at the hospital after fasting overnight. Upon arrival, the subject had an intravenous catheter inserted for blood sampling. Prior to the administration of the peppermint oil capsule, several baseline measurements were taken; a venous blood sample was taken to determine baseline plasma menthol glucuronide, an evaluation of baseline side effects was conducted, and blood pressure/heart rate were measured. At $t = 0$, the study medication was administered with a 200-mL glass of water. Consequently, at $t = 1$, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, and 24 h, venous blood sampling, side effect evaluation, and blood pressure/heart rate measurements were repeated. Two hours after capsule intake, a standardized breakfast was provided (two sandwiches with cheese and cucumber, glass of milk, 384 calories in total). Lunch and dinner were subsequently provided at $t = 6$ h and $t = 10$ h after capsule intake, respectively. The last measurement before midnight took place at $t = 14$ h, after which the intravenous cannula was removed and the subject was allowed to return home. Participants returned to the hospital for the last measurements at $t = 24$ h. Throughout the complete study, volunteers were instructed to report any side effects. A telephonic evaluation took place between both test periods.

Primary and Secondary Outcomes

The primary outcome was T_{max}, time to reach peak menthol glucuronide concentration in plasma. The secondary outcomes were T_{lag}, time to reach a menthol glucuronide concentration of 45 μg/L; AUC, area under the plasma concentration–time curve; C_{max}, peak menthol glucuronide concentrations; $T_{1/2}$, elimination half-life; side effects and tolerability, as determined by a side effect questionnaire and blood pressure/heart rate measurement.

Pharmacokinetic Analysis

The pharmacokinetic profile of small intestinal and ileocolonic release peppermint oil capsules was determined by menthol glucuronide analysis in the blood. In total, 14 venous blood samples (± 8 mL per time point) were collected in heparinized tubes at the time points

mentioned above. Within 2 h after collection, samples were centrifuged at 3120 rpm for 10 min at 4 °C and plasma supernatants were stored at − 80 °C until assayed. Samples were analyzed for menthol glucuronide concentration, the primary metabolite of L-menthol, by a 15-h incubation at 37 °C with beta-glucuronidase and using gas chromatography mass spectrometry (GC/MS); the method has been described in detail elsewhere [34, 35]. A detection limit of 5 µg/L applied. Menthol glucuronide concentrations are expressed in micrograms per liter.

Statistical Analysis

A power calculation was performed (two-sided $\alpha = 0.05$; power = 0.80; Sd = 109; minimal detectable difference in means = 150 min); at least seven subjects were needed to complete the study to reliably demonstrate a significant difference in time to reach peak menthol glucuronide concentration in the plasma (T_{max}), between the small intestinal versus the novel ileocolonic release peppermint oil capsules. Anticipating one dropout, the aim was to include eight participants.

Statistical analyses were carried out using IBM SPSS statistics 23.0 (Chicago IL, USA) and GraphPad Prism 6.0 (La Jolla, CA, USA) for Macintosh. As the sample size was small ($N = 8$), data were analyzed using non-parametric tests. T_{max}, T_{lag}, and C_{max} values were determined directly from the plasma concentration–time profiles for each subject and were analyzed for comparison by the Wilcoxon-signed rank test. AUCs and $T_{1/2}$ were calculated by pharmacokinetic software MWPharm 3.80 (Mediware) using the log-linear trapezoidal rule (non-compartmental analysis). AUCs and C_{max} were logarithmically transformed and a 90% CI interval was calculated for the log-transformed parameter ratios (small intestinal/ileocolonic release) to assess bioequivalence. A p value less than 0.05 was considered statistically significant. There were no missing data.

Safety and Tolerability Analysis

Subjects were monitored for adverse events by direct observation during the first 14 h after peppermint oil administration and again for 1 h after 24 h. During both test periods, participants completed a questionnaire regarding adverse events and tolerability at the 14 time points mentioned above. In addition, vital signs were reviewed at these 14 time points. In between test periods and after the last test period, volunteers were telephoned to inquire about possible adverse events.

RESULTS

In Vitro GISS Experiment

In vitro analysis of the newly produced ileocolonic release peppermint oil capsules in the GISS showed that the actual release of the peppermint oil was postponed until the last phase (colon). Part of the coating remained intact and contained a small residual amount of

Table 2 Results of ileocolonic release peppermint oil capsules in in vitro dissolution test (GISS)

Phase	Gastrointestinal segment	Capsule appearance	Capsule location in dissolution vessel	Oil observed on surface buffer solution
I	Stomach	Intact	Bottom	None
II	Jejunum	Intact, minor cracks in coating	Bottom	Slight amount
III	Ileum (distal)	Intact, small cracks in coating	Bottom	Small amount
IV	Colon (proximal)	Open—small residual amount of oil inside	Floating on surface buffer solution	Large amount

GISS gastrointestinal simulation system, *PO* peppermint oil

peppermint oil. For further details and photographic documentation of these results, please refer to Table 2 and the supplementary material.

Phase I Human Trial

Eight healthy volunteers were screened, included, and randomly assigned to a specific treatment order. All participants were between 20 and 65 years old (median 22.2, IQR 20.8–28.8). See Table 3 for baseline characteristics. All participants completed the study.

Menthol Glucuronide Levels

Baseline menthol glucuronide concentrations were all below the detection limit, except for one subject on the first test day who had a concentration of 5.79 µg/L. These low levels were considered evidence that the instructions given regarding avoidance of menthol containing products were sufficient.

The pharmacokinetic parameters of 182 mg small intestinal and 182 mg ileocolonic release peppermint oil are shown in Table 4. For an overview of plasma concentration–time curves per individual subject, see Fig. 2 and the appendix/supplementary material. T_{max} of the ileocolonic release peppermint oil capsules was significantly longer in all participants compared to T_{max} of small intestinal release peppermint oil with a median (IQR) of 360 min (360–405) versus 180 min (120–180) respectively, $p < 0.05$ ($p = 0.010$). Median difference in T_{max} between both formulations in individual participants was 180 min (IQR 180–240, 95% CI 180–140). T_{lag} was significantly delayed in ileocolonic release peppermint oil, with a median (IQR) of 225 (204–284) versus 37 (6–65) min, $P < 0.05$ ($p = 0.012$). In addition, $AUCs_{0-24h}$ differed significantly between the ileocolonic and the small intestinal release capsules, with a median (IQR) of 2331 µg h/L (2006–2510) and 2623 µg h/L (2471–2920) respectively, $p < 0.05$ ($p = 0.017$). No significant differences were found in C_{max} ($p = 0.28$) and $T_{1/2}$ ($p = 0.16$) of either capsule. Mean ratios of log-transformed AUCs and C_{max} of 1.02 (90% CI 1.01–1.04) were found.

Adverse Events and Tolerability

No serious adverse events occurred during the study nor during one week of follow-up. Adverse events were reported in five subjects, but were all mild and transient. Adverse events are shown in Table 5.

DISCUSSION

This is the first study to investigate a novel ileocolonic release peppermint oil formulation

Table 3 Summary of participant demographic and baseline characteristics

	Total ($N = 8$)	Small intestinal release PO on first test day ($N = 4$)	Ileocolonic release PO on first test day ($N = 4$)
Female sex, *N* (%)	4 (50)	1 (25)	3 (75)[a]
Age, years	22.2 (20.8–28.8)	24.1 (21.2–55.0)	21.7 (20.6–27.9)
BMI, kg/m^2	21.5 (20.2–23.0)	21.7 (18.5–23.8)	21.5 (20.6–22.2)
Height, cm	177 (171–183)	181 (177–192)	173 (168–177)
Weight, kg	65.5 (64.0–72.0)	71.0 (65.3–79.8)	64.0 (61.8–66.3)

Data are presented as median (IQR), unless otherwise noted. Differences were tested using Mann–Whitney *U* test for non-parametric data and chi-square or Fisher's exact for parametric data

N number, *IQR* interquartile rage, *BMI* body mass index

[a] Significant difference in gender between the group receiving small intestinal peppermint oil (PO) on the first test day and the group receiving ileocolonic release PO on the first test day ($p < 0.05$). $N = 8$ (total group)

Table 4 Pharmacokinetic parameter results; small intestinal release and ileocolonic release peppermint oil

	Small intestinal release peppermint oil	Ileocolonic release peppermint oil	Ratio LN-transformed parameter (90% CI)
T_{max} (min)			NA
Arithmetic mean (SEM)	165 (15)	375 (19)[a]	
Median (IQR)	180 (120–180)	360 (360–405)[a]	
Geometric mean (SEM)	160 (15)	372 (19)[a]	
T_{lag} (min)			NA
Arithmetic mean (SEM)	38 (12)	241 (18)[a]	
Median (IQR)	37 (6–65)	225 (204–284)[a]	
Geometric mean (SEM)	22 (12)	237 (18)[a]	
AUC_{0-24} (μg h/L)			1.02 (1.01–1.04)
Arithmetic mean (SEM)	2664 (84)	2246 (118)[a]	
Median (IQR)	2623 (2471–2920)	2331 (2006–2510)[a]	
Geometric mean (SEM)	2655 (84)	2222 (118)[a]	
C_{max} (μg/L)			1.02 (1.01–1.04)
Arithmetic mean (SEM)	817.9 (90)	558.0 (100)	
Median (IQR)	702 (644–1020)	563.6 (268–849)	
Geometric mean (SEM)	788.2 (90)	487 (100)	
$T_{1/2}$ (h)			NA
Arithmetic mean (SEM)	9.2 (1.4)	6.4 (0.6)	
Median (IQR)	7.7 (7.0–10.8)	6.1 (5.1–7.4)	
Geometric mean (SEM)	8.6 (1.4)	6.2 (0.6)	

Differences between both peppermint oil capsules were tested using Wilcoxon signed-rank test

LN natural log, *CI* confidence interval, T_{max} time to reach maximum plasma concentration, *SEM* standard error of the mean, *IQR* interquartile range, T_{lag} time to reach a menthol glucuronide concentration of 45 μg/L, *AUC* area under the concentration–time curve, C_{max} maximum concentration, $T_{1/2}$ time required for the plasma concentration to reach half of its original value (elimination half-life), *NA* non-applicable

[a] Significant difference between small intestinal release and ileocolonic release peppermint oil ($p < 0.05$)

and to compare its pharmacokinetic parameters, safety, and tolerability with the small intestinal release peppermint oil formulation currently available. Our results demonstrate that ileocolonic release peppermint oil soft capsules have a significantly delayed lag time (T_{lag}) and reach their peak concentration significantly later (T_{max}) than small intestinal release peppermint oil capsules. These findings point to the release of peppermint oil in a more distal part of the human gastrointestinal tract, the colon most presumably. Although different in release kinetics, a single dose of both formulations of peppermint oil can be considered bioequivalent regarding exposure in healthy volunteers, because the 90% confidence interval (CI) for the log-transformed parameter ratios (small intestinal/ileocolonic release) is between

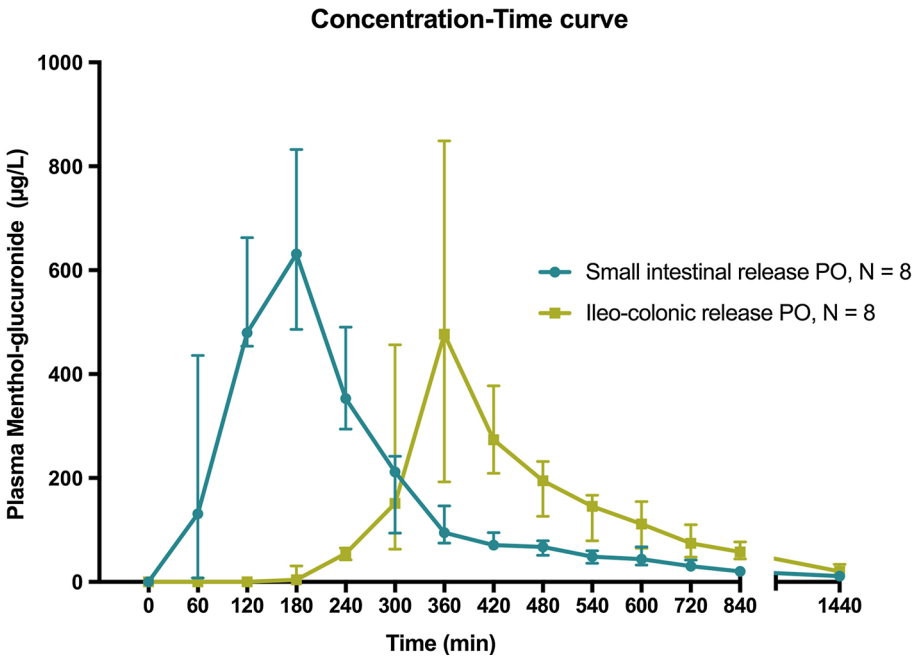

Fig. 2 Concentration–time curve: menthol glucuronide concentration was measured in eight healthy volunteers ($N = 8$) in µg/mL at 14 time points after both peppermint oil (PO) capsule administrations. The ileocolonic release PO has a significantly elongated time to reach the maximum plasma concentration (T_{max}). Circles and squares represent median plasma glucuronide levels + interquartile range (IQR)

Table 5 Adverse events occurring after administration of a single 182 mg peppermint oil dose

	Small intestinal release PO ($N = 8$)	Ileocolonic release PO ($N = 8$)
Acid regurgitation, *N* (%)	1 (12.5)	0 (0)
Fecal urgency, *N* (%)	1 (12.5)	0 (0)
Headache, *N* (%)		
Mild	0 (0)	2 (25)
Moderate/severe	0 (0)	0 (0)
Altered fecal odor, *N* (%)	0 (0)	2 (25)
Vital sign abnormalities, *N* (%)	0 (0)	0 (0)

PO peppermint oil, *N* number. $N = 8$ (total group). Altered fecal odor implied a peppermint oil odor

the 80% and 125% confidence interval, the standard bioequivalence criterion as stated in the FDA and EMA guidelines on the investigation of bioequivalence [30, 31].

Several authors have previously reported on the pharmacokinetic parameters of small intestinal release peppermint oil, often referred to as enteric coated capsules: White et al. found a mean T_{max} of 5 and 2.8 h for menthol glucuronide after three Colpermin® or Mintec® capsules were taken, containing 0.2 ml peppermint oil each and a mean L-menthol concentration of 110 mg and 117 mg, respectively [19]. Mascher et al. found a mean T_{max} of 3 h and a mean menthol C_{max} of 1196 ng/ml after two Enteroplant® capsules were taken, containing 90 mg of peppermint oil each [33]. Our findings for the small intestinal release peppermint oil capsules are in line with those of both White et al. and Masher et al. in terms of T_{max} found, although the C_{max} differed slightly. A possible explanation for this modest discrepancy is that different small intestinal release formulations of peppermint oil, produced by different manufacturers, produced from a different harvest of mint leaves, were used. L-Menthol concentrations in peppermint oil are known to vary between 30% and 55% [32]. When taken orally,

L-menthol is rapidly metabolized to menthol glucuronide, which can be measured successfully in blood plasma by GC/MS [34]. Consequently, the menthol glucuronide is excreted in urine [9].

The menthol glucuronide concentration was not measured directly in the small intestine and colon because of the practical difficulties of an ileocolonic intubation. Unlike other studies that have compared peppermint oil formulations, however, we think pharmacokinetic parameters could be compared more reliably as both formulations used here contained the same amount of L-menthol; the ileocolonic release peppermint oil capsules were created by overencapsulation of small intestinal release peppermint oil capsules from the same manufacturer [19]. We presume that the difference in T_{max} found between the small intestinal and ileocolonic release peppermint oil reflects the longer transit needed to reach the colon.

It should be also noted that our study showed large interquartile ranges in plasma menthol glucuronide levels, indicating high intersubject variability. Measures taken to decrease any variability were standardized meals and snacks, an overnight fast, and the complete abstinence from caffeine, alcohol, smoking, and medicines affecting gastrointestinal function in a predefined period prior to drug administration. The variability found can therefore probably be explained by normal biological variation in gastrointestinal transit times, and polymorphic variation in cytochromes P450 (CYP) enzymes, which are known to facilitate L-menthol metabolism [36–39].

The delayed peak concentration of the novel ileocolonic release peppermint oil and, thus, more distal exposure to peppermint oil are not only expected to increase therapeutic efficacy, but they are also expected to lead to a different and possibly milder spectrum of adverse events, as there is less upper gastrointestinal but more distal gastrointestinal exposure to peppermint oil. This pilot study has examined possible adverse events after only a single dose of peppermint oil and was not powered to draw any conclusions regarding adverse events since it only included eight volunteers. In addition to evaluating short-term adverse events, future studies should also evaluate the long-term adverse events occurring when peppermint oil capsules of 182 mg are taken three times daily, the dosage identified by previous studies as being effective in IBS patients and the industry norm [20, 22, 24, 40].

As discussed above, there are indications that peppermint oil has a direct local antinociceptive effect in the intestine through an interaction with TRP channels. When peppermint oil makes contact with the skin or oral membranes, a general cooling sensation is induced through stimulation of the TRPM8 (transient receptor potential melastin-8) receptor [41]. Interestingly, experimental research in murine models suggests that L-menthol, through stimulation of the TRPM8 receptor, may be able to cross-desensitize the TRPV1 (transient receptor potential vanilloid-1) receptor. TRPV1 is a pronociceptive receptor, well known for its involvement in animal models of visceral hypersensitivity and its upregulation in the colon mucosa of IBS patients [13, 42], indicating a role in pain generation in IBS. Together with its role in thermosensation, TRPM8 is believed to play a role in protective mechanisms in states of intestinal inflammation [43]. Similar to the stimulation of TRPM8, menthol may also be able to stimulate the TRPA1 (transient receptor potential ankyrin-1) receptor, another TRP channel suggested to contribute to visceral pain. TRPV1, TRPA1, and TRPM8 receptors are probably co-expressed on ileocolonic mucosal afferents [11, 44], suggesting a complex and incomprehensively understood interaction between these receptors that leads to the analgesic effect of peppermint oil. We hypothesize that the ileocolonic release of peppermint oil enhances the therapeutic efficacy as the application specifically results in increased exposure of the ileocolonic mucosal afferents described. Moreover, the antispasmodic effect and, thereby, alleviating effect of peppermint oil performs equally well, if not better, when peppermint oil is applied to the colon locally; it has been applied intraluminally in endoscopic practice to decrease pain caused by the procedure and to enhance the field of view during the endoscopy through the suppression of peristalsis [45–47]. This pharmacokinetic study served

as proof of concept study and we are currently conducting a randomized, placebo-controlled trial in IBS patients to compare the efficacy of small intestinal and ileocolonic release peppermint oil in IBS patients (NCT02716285). This study should confirm whether peppermint oil capsules with an ileocolonic release do indeed attenuate abdominal pain in IBS patients.

A potential limitation of this study is that L-menthol was the only ingredient of peppermint oil taken into account; other constituents of peppermint oil include menthone, cineole, menthyl acetate, isomenthone, menthofuran, limonene, pulegone, carvone, and isopulegol [32, 48]. These could potentially contribute to clinical effects, but were not measured in the plasma samples. For example, in addition to the antispasmodic effect, peppermint oil has been shown to inhibit 5-HT_3 in the human colon [15]. This inhibitory effect could only be partly accounted for by L-menthol in another experimental study [49], suggesting the involvement of one or more of the other ingredients mentioned above. Furthermore, the toxic compounds known to be present in peppermint oil in very low quantities—in equal dosages for ileocolonic release and small intestinal release peppermint oil—were also not measured. Pulegone and menthofuran normally appear in peppermint oil in doses of between 1–9% and less than 4.0% [32] and could potentially harm chronic peppermint oil users. Some animal studies reported toxicity due to pulegone and menthofuran; high dosages in rats were associated with hepatocyte vacuolization, liver necrosis, and possibly cyst-like spaces in the cerebellum [7]. Nevertheless, when taken in the recommended dosages, the EMA and FDA consider peppermint oil to be generally safe. Even if the EMA states that no confirmed cases of liver damage due to peppermint oil usage have been reported [50], they do advise against the continuous use of peppermint oil for longer than 3 months [51]. It remains to be elucidated whether this advice is substantiated—no studies have assessed the long-term effect and thus no studies have revealed damage occurring after 3 months—but further research into this topic is certainly desired.

Of note is that half of the participants were male, whereas the male/female ratio in IBS patients is usually estimated to be 1:2 [52]. Ideally, future research should include IBS patients who may experience altered motility and/or altered transit times and should preferably also investigate relatively more female subjects. Although large effects of sex on pharmacokinetic parameters are not expected, factors such as menstrual cycle and lower body weights, and thereby smaller distribution volumes, higher body fat percentages etc. could be of influence [53].

CONCLUSIONS

A novel ileocolonic release peppermint oil formulation has been developed to decrease upper gastrointestinal side effects associated with small intestinal release peppermint oil. This study provides evidence that the ileocolonic release peppermint oil has a significantly delayed peak menthol glucuronide concentration, pointing to a more delayed and therefore more distal intestinal release of peppermint oil. The ileocolonic release may enhance therapeutic efficacy as it results in increased exposure to the colonic mucosal afferents and decrease adverse events. A randomized placebo-controlled trial (RCT) investigating the efficacy of small intestinal and ileocolonic release peppermint oil in IBS patients has been initiated and is currently ongoing. This RCT is based on the pharmacokinetic data from the present study.

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Compliance with Ethics Guidelines. All procedures performed in in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants gave a written informed consent prior to participation.

Data Availability. The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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