BMJ Open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2015-008375
Article Type:	Research
Date Submitted by the Author:	31-Mar-2015
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Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	PAEDIATRICS, Paediatric gastroenterology < PAEDIATRICS, THERAPEUTICS

Systemic Exposure to Menthol Following Administration of Peppermint Oil to Paediatric Patients

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Key Words: Pharmacology, Therapeutics, Paediatrics

Word count: 3251

Figures: 1

Tables: 2

Disclosure / Conflict of Interest: None of the authors have any apparent conflict of interest related to the test agent used in this clinical trial and the company that provided it.

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ABSTRACT

Objective: Peppermint oil (PMO) has been used to treat abdominal ailments dating to ancient Egypt, Greece and Rome. Despite its increasing paediatric use as an IBS treatment, the PK of menthol in children given PMO has not been explored.

Design and Setting: Single-site pilot study of menthol PK following a single 187 mg dose of PMO. Subjects with pediatric Rome II defined IBS (n=6, male and female, 7-15 yr of age) were enrolled. Blood samples were obtained before PO administration and at 10 discrete time points over a 12 hr post-dose period. Menthol was quantitated from plasma using a validated GC-MS technique. Menthol PK parameters were determined using a standard non-compartmental approach.

Results: Following a dose of PMO, a substantial lag time (range 1-4 hr) was seen in all subjects for the appearance of menthol which in turn produced a delayed time of peak (Tmax = 5.3±2.4 hr) plasma concentration (Cmax = 698.2±245.4 ng/ml). Tmax and Tlag were significantly more variable than the two exposure parameters; Cmax, mean residence time (MRT) and total area under the curve (AUC=4039.7±583.8 ng/ml*hr) which had a coefficient of variation (CV) of <20%.

Conclusions: Delayed appearance of menthol in plasma after oral PMO administration in children is likely a formulation-specific event which, in IBS, could increase intestinal residence time of the active ingredient. Our data also demonstrate the feasibility of using menthol PK in children with IBS to explore the developmental impact of concentration vs. exposure relationships for menthol in this disorder.

ARTICLE SUMMARY

Strength of the findings:

- An initial description of the concentration vs. time relationship for menthol following administration of a proprietary peppermint oil (PMO) formulation being used to treat paediatric patients with Irritable Bowel Syndrome.
- A basis for the design of future paediatric investigations of PMO designed to characterize the exposure-response relationship for menthol in children with Irritable Bowel Syndrome.

Limitations of the study:

- A small study cohort which, while informative regarding the clinical pharmacology of menthol from PMO, may not reflect the true population variability in the dose vs. exposure relationship.

INTRODUCTION

Peppermint oil (Menthae piperitae aetheroleum) is obtained by steam distillation from the fresh leaves of peppermint (*Mentha piperita* L.). The major constituents of peppermint oil (PMO) include the terpenes (-) menthol (30-50%), (-) menthone (14-32%), (+) isomenthone (1.5-10%), (-) menthyl acetate (2.8-10%), (+) menthofuran (1.0-9.0%), and 1,8-cineol (3.5-14%). As reviewed by Grigoleit and Grigoleit [1], the pharmacologically active ingredient of PMO is menthol which in nature, exists as a pure stereoisomer (1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexanol).

Through its ability to act as a calcium antagonist, menthol appears to have a spasmolytic effect in the gastrointestinal tract. Consequently, PMO has been used to treat abdominal ailments dating to ancient Egypt, Greece and Rome [2] and anecdotal evidence of its purported efficacy abounds to this day. A review and several meta-analyses of randomized, double blind, placebo controlled trials in adults have demonstrated that PMO is effective in reducing abdominal pain in patients with irritable bowel syndrome (IBS). [1, 3-7] However, in children with IBS, there is a dearth of information restricted to a single small (n=42), double blind, placebo controlled trial.[7] Nonetheless, the use of PMO as an adjunctive measure to treat children with IBS continues to evolve in clinical practice despite the lack of information (e.g., age-dependent disposition characteristics; a defined dose vs. concentration vs. effect relationship, developmental differences in pharmacodynamics). Thus, dosing remains empiric in both paediatric patients and adults where a range in daily doses of more than 2.5-fold has been reported. [1]

Given the increasing use of PMO in pediatric patients with IBS and the potential for developmental changes to influence both the pharmacokinetics and pharmacodynamics of drugs [8], we conducted a pilot pharmacokinetic (PK) study of menthol following the administration of a proprietary formulation of PMO in a cohort of paediatric patients with IBS. The primary objective of this study was to examine the

concentration vs. time profile of menthol following a single PMO dose and to explore apparent associations between PMO dose and menthol pharmacokinetics in this "target" population.

METHODS

Patients

We performed a single-site, proof-of-principle pilot study of menthol pharmacokinetics (PK) following a single 187 mg oral dose of (Colpermin®; Tillotts Pharma, Rheinfelden, Switzerland). Patients with paediatric Rome III defined IBS (n=6) who had no intercurrent illness or recent change in IBS symptoms were enrolled into a protocol approved by the Baylor College of Medicine Institutional Review Board. Children initially were identified by review of medical records. They were screened by telephone to be sure they qualified and that IBS symptoms were current. They were otherwise healthy. Absence of illness was assessed via history and physical examination at the time of the study visit. No participant was receiving therapeutic medications and/or natural products known to influence the activity of either hepatic drug metabolism (i.e., no enzyme inducers or inhibitors) or renal drug clearance. Finally, other than an existing diagnosis of IBS, no study participant had a history of an abnormality or surgery of the gastrointestinal tract.

All study participants were enrolled by informed parental consent and when appropriate (e.g. > 6 years of age), by patient assent. Informed consent was documented prior to performing any study-related procedure.

Drug administration, sampling and sample handling

The clinical phase of this study was conducted in strict accordance with Good Clinical Practice principles. Prior to being admitted to the Metabolic Research Unit (MRU) at the Children's Nutrition Research

Center, study participants were fasted from midnight immediately preceding the morning of admission (i.e., overnight). Following the history, physical examination and recording of vital signs (temperature, pulse rate, respiratory rate, seated blood pressure), a venous cannula (21 gauge) was aseptically placed in a large vein either in the dorsum of the hand or on the volar surface of the lower arm to facilitate obtaining repeated blood samples to support the PK objectives of the study. The cannula was secured and its patency maintained through periodic flushing of the dead space with sterile 0.9% sodium chloride.

At approximately 0900, subjects received a single oral dose of PMO given as an available, proprietary, non-prescription product (Colpermin®, each capsule containing approximately 83.0 mg of menthol as a constituent of PMO) followed by 120 mL of water at 25°C. Immediately prior to administration of the test article, a blood sample (2.0 mL) was obtained. After PMO administration, repeated blood samples (2.0 mL each) were obtained directly into green top glass tubes containing sodium heparin (Vacutainer®, Becton Dickinson, East Rutherford, NJ) at the following post-dose time points: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours. At 2 hours after PMO administration, participants were given a standardized meal. Throughout the 12-hour study period, participants were restricted from any strenuous physical exercise. After completion of the final (12-hour) sample, vital signs were reassessed, the venous cannula was removed and the insertion site evaluated for redness, swelling and/or bruising. Study participants were then discharged from the MRU and received a follow up call from a clinical research coordinator approximately 24 hours thereafter to specifically assess/evaluate any potential adverse effects.

Immediately following collection of a given blood sample, it was mixed by gentle inversion and the tube immediately placed at 4°C. At 4-hour intervals, blood samples were centrifuged (2,500 x g for 10 minutes at 4°C). The resultant plasma from each tube then was immediately transferred into a clean,

screw capped polypropylene tube and immediately frozen at -80°C. Samples were maintained at 2-8°C while handling so as to minimize evaporative loss of any volatile compounds contained therein.

Analytical

Menthol in plasma was measured by gas chromatography mass spectrometry (GC-MS) as described in previous methods with several minor modifications.[9, 10] Briefly, to 0.5 mL of the patient's heparinized plasma or controls or calibrators, 20 µL internal standard (menthol-d4, 10 µg/mL), 25 µL β-glucuronidase (90,000 U/mL), and 10 µL sodium acetate buffer (pH 4.8) were added. The mixture was incubated overnight in air tight tubes in 37^0C water bath, and to each tube, 150 µL 0.4 M phosphate buffer (pH 4.8) was added. Menthol was extracted in 0.5 mL methylene chloride by rocking the tubes for 5 minutes. The mixture was centrifuged at 2000 g for 5 minutes. The methylene chloride layer was transferred to an auto-sampler vial and a 2 µL extract was injected onto GC-MS (Agilent Technologies, Santa Clara, CA) installed with ZB-1MS 15m x 250um x 0.25 µm column. Ions (m/z) monitored for quantification and identification were138, 123, 95 for menthol and 142, 127, 99 for menthol-d4. The data were analyzed using Target Software (Thru-Put Systems, Orlando, FL). The quantitative ions were used to construct standard curves of the peak area ratios (calibrator/internal standard pair). The assay was linear within the range of concentrations evaluated 5-1000 ng/mL (r^2>0.99). For all standards the intra- and inter-assay coefficient of variations (CVs) were <10%.

Pharmacokinetic data analysis

Menthol plasma concentration versus time data were evaluated using a model independent approach. Individual Cmax and Tmax were obtained by direct examination of the plasma concentration versus time profile. The area under the plasma concentration versus time curve during the sampling period (AUC_{0-n}) was calculated using the mixed log-linear method where "n" refers to the final sampling time with

quantifiable menthol concentrations. The terminal elimination rate constant (λz) was estimated using an iterative least-squares regression algorithm. The total AUC (AUC_{tot}) was extrapolated to infinity by dividing λz into the predicted concentration at the end of the sampling interval. As a subset of our patients (n=3) did not have > 3 post-Cmax plasma concentrations, there was significant uncertainty associated with the estimation of λz and consequently the pharmacokinetic parameters that rely on the terminal rate constant [e.g. AUC to infinity ($AUC_{0-\infty}$), apparent oral clearance (Cl/F), apparent volume of distribution at steady-state (Vss/F)] are not reported. Menthol pharmacokinetic data were examined using standard descriptive statistics. All analyses were performed in Kinetica™ version 5.1 (Thermo Scientific) and SPSS version 20 (IBM SPSS).

RESULTS

All six participants completed the study without experiencing any adverse events. The study cohort consisted of 4 female and 2 male patients. Their age range was 7 to 12 years (10.3±1.9 yr.) and their body weights ranged from 26.6 to 58.2 kg (45.2±11.5 kg). None of the participants had a height or weight which was outside of the 5^{th} to 95^{th} percentile for age.

The composite (mean±95% confidence limits) and individual plasma menthol concentration vs. time data are shown in Panels A and B of the Figure, respectively. Considerable intersubject variability in the plasma concentration vs. time profiles was apparent with all participants in the cohort. Individual pharmacokinetic (PK) parameters for menthol are provided in Table 1. The time of appearance (Tmax) for apparent peak plasma concentrations (Cmax) ranged from 2.5 to 8 hours following a lag time (Tlag) which ranged from 1.5 to 4 hr. Absolute Cmax values ranged from 458 to 1056 ng/mL which when corrected to a weight adjusted menthol dose received by each child, ranged from 255 to 470 ng/mL per 1 mg/kg (i.e., an approximate 1.8-fold difference). The dose normalized systemic menthol exposure

reflected by the area under the plasma concentration vs. time curve (AUC per mg/kg dose) varied over an approximate 2-fold range (1464.1 to 2941.1 ng/mL*hr). Given the marked delay in the appearance of menthol in plasma after a PMO dose, sufficient post-peak plasma concentrations to reliably estimate an apparent elimination rate constant (λz) and half-life were not available. Consequently, the non-compartmental parameter mean residence time (MRT; a time representing elimination of approximately 60% of a given dose from the body) was determined using statistical moment theory and ranged from 6.4 to 9.4 hours (Table 1).

Table 1: Individual Menthol Pharmacokinetic Parameters

Participant	Menthol dose (mg/kg)	Cmax (ng/mL)	Cmax (ng/mL per mg/kg dose)	Tmax (h)	Tlag (h)	AUClast (ng/mL*h per mg/kg dose)	AUCtot (ng/mL*h per mg/kg dose)	MRT (h)
MentPK-01	1.4	628	445.1	2.5	1.5	2941.1	3199.2	7.0
MentPK-02	1.5	474	325.0	6	1	2045.6	2245.9	7.5
MentPK 03	2.0	936	470.5	4	2	1951.4	2137.1	6.4
MentPK 04	3.1	1056	342.1	8	4	1198.4	1350.5	9.1
MentPK 05	1.9	637	332.8	3	1.5	2107.2	2434.8	7.4
MentPK 06	1.8	458	255.5	8	2	1464.1	1879.5	9.4

Abbreviations include: Cmax, apparent peak plasma concentration; Tmax, time of Cmax; Tlag, apparent lag time between PO administration and appearance of menthol in plasma; AUC, area under the plasma concentration vs. time curve and MRT, mean residence time.

A summary of the pharmacokinetic parameters (mean, SD and 95% confidence intervals) is provided in Table 2. As reflected by the values for the coefficient of variation for each of the parameters, the least variability was observed for MRT (CV = 15.4%) and AUCtot (14.4%).

Table 2: Summary of Menthol Pharmacokinetic Parameters

Parameter	mean ± SD	95% CI	CV%
Cmax (ng/mL)	698.2 ± 245.4	[501.8, 894.6]	35.1
Cmax (ng/mL per mg/kg)	361.8 ± 80.8	[297.2, 426.5]	22.3
Tmax (h)	5.3 ± 2.4	[3.3, 7.2]	45.3
Tlag (h)	2.0 ± 1.0	[1.2, 2.8]	50.0
AUClast (ng/mL*h)	3562.0 ± 616.7	[3068.5, 4055.4]	17.3
AUC last (ng/mL*h per mg/kg dose)	1951.3 ± 602.8	[1468.9, 2433.7]	30.9
AUCtot (ng/mL*h)	4039.7 ± 583.8	[3572.6, 4506.9]	14.4
AUC total (ng/mL*h per mg/kg dose)	2207.8 ± 613.8	[1716.7, 2698.9]	27.8
%AUCextrapolated	12.1 ± 5.3	[7.8, 16.3]	43.8
MRT (h)	7.8 ± 1.2	[6.8, 8.8]	15.4

Abbreviations: SD, standard deviation; CI, confidence interval; CV, coefficient of variation. Pharmacokinetic parameter abbreviations contained in footnote of Table 1

DISCUSSION

The putative active ingredient contained in PMO is menthol, a plant-derived, semi-volatile monoterpene. The clinical pharmacology of menthol, with an emphasis on its interaction with sensory neurons (TRP channels), has been recently reviewed.[11] The disposition and pharmacokinetics of menthol derived from the ingestion of peppermint oil in adults also has been reported previously [12-14] as have the pharmacokinetics of *L*-menthol administered directly to the upper gastrointestinal

tract.[15] In a cohort of 12 adults administered an oral dose of *L*-menthol (10 and 100 mg), the drug was rapidly metabolized with conversion to a menthol glucuronide which could be measured in plasma and urine.[14] In a similar study of 16 adults reported by Mascher, et al. [13], the apparent time of peak plasma concentration (Tmax) of methanol (measured by GC/MS) following oral administration of a 180 mg dose of enteric coated PMO occurred at approximately 3 hours following drug administration. The decline of methanol levels in the plasma was rapid with an average apparent elimination half life of approximately 3.5 hours.

Recent studies have shown that much of the clearance of menthol from plasma is associated with the activity of CYP2A6 [16], a polymorphically expressed cytochrome P450 isoform that is responsible for the biotransformation of other xenobiotics such as the primary nicotine metabolite, cotinine [17] and the commonly used antimicrobial agent, metronidazole.[18] In the case of cotinine, Dempsey, et al. [17] demonstrated that *CYP2A6* genotype (a surrogate for CYP2A6 phenotype), as opposed to age, was the major determinant of plasma elimination half life in infants and children. To date, the PK of menthol have not been evaluated or reported in a pediatric patient cohort. Thus, a significant data gap exists with regard to the therapeutic use of PMO in children and adolescents.

Despite data from only a single available pediatric trial of PMO in children with IBS [7], the substance is being increasingly used either as adjunctive or primary treatment of patients with this disorder based on anecdotal evidence of symptomatic improvement. It is not known whether the potential analgesic effects of menthol in patients with IBS have a central [19] and/or local basis. In contrast, the antispasmodic effects appear to be modulated locally as demonstrated by Hiki, et al. [15] in a study where *L*-menthol was directly sprayed onto gastric mucosa. It is for this reason that the Colpermin®

formulation of PO is an enteric-coated tablet designed to release the active ingredients at a pH encountered in the small intestine (i.e.,> 6.5).[20]

As illustrated in the Figure, the appearance of menthol in the plasma of all subjects in our cohort was delayed with an average lag time of 2 hours; a finding consistent with aggregate data in adults summarized by the manufacturer of our test article.[20] This finding is supported to some extent by two earlier studies of the Colpermin® product which examined the pharmacokinetics of menthol in adults using urinary menthol excretion data [21,22] and a more recent adult study which examined menthol concentrations in plasma following the administration of a different enteric-coated PMO product (Enteroplant®, Spitzner Pharmaceuticals, Ettlingen, Germany).[23] Unlike the plasma concentration profile reported by Mascher, et al. [23], the mean plasma menthol concentration vs. time data in our paediatric study cohort (Figure; top panel) demonstrated an apparent prolonged absorption time with apparent peak plasma concentrations (Tmax) occurring between 2.5 and 8 hours post-dose (Table 1). Reasons for the delayed Tmax likely reside with formulation-specific factors (i.e., delayed release) and potentially, enterohepatic recirculation, as menthol undergoes biotransformation by UDP-glucuronosyltransferase 2B7 (UGT2B7).[24, 25] The apparent post-Tmax increase in plasma methanol concentrations in several of our study participants (Figure, bottom panel) suggests the presence of enterohepatic recirculation of PMO.

In contrast to the adult data reported by Masher, et al. [23], we were not able to reliably estimate the apparent terminal elimination rate constant (λz) for menthol in our entire patient cohort; namely, patients 2, 4 and 6 where ≤ 2 post-Cmax plasma concentrations were available for estimation of the parameter. It was for this reason that we chose to use a non-compartmental approach to describe menthol pharmacokinetics and to examine mean residence time (MRT) as a parameter reflecting drug

elimination. As noted above, our inability to produce a reliable estimate of λz precluded our reporting reliable estimates for the apparent plasma clearance and/or volume of distribution for menthol. Also, given the prolonged absorption for menthol in our cohort (Figure), estimation of λz may have produced an errant result consequent to the phenomenon of the "flip-flop model" which occurs when the rate of absorption and/or distribution is slower than the rate of true drug elimination.[26] Nonetheless, based upon known patterns of ontogeny with regard to the activity of UGT isoforms [8], we would not expect that the apparent elimination half life of menthol in our study participants (i.e., range from 1.6 to 3.0 hr in 3 patients with reliable λz estimates) would be similar to values for this parameter (3.5 to 4.4 hours) previously reported from adults.[23] A more accurate estimate of λz would have been available in our paediatric cohort had we previously had knowledge of the prolonged menthol absorption profile. This would have enabled us revise our blood sampling paradigm to cluster observations between 6 and 18 hours post-dose and to extend the sampling interval to 24 hours.

Despite considerable variability in the plasma menthol concentration vs. time data in our patients (Figure), the values for the AUCtot and MRT had the smallest coefficients of variation associated with them (Table 2). This relatively close agreement in the PK parameters suggested by the coefficients of variation would seemingly support that despite the presence of allelic variants for CYP2A6 [17] and UGT2B7 [25] in the general population, the concentration vs. time data for menthol in our cohort did not contain an "outlier" which could have been contributable to one or more of the participants having inherited an allelic variant for either enzyme which conveys reduced enzyme activity. The potential significance of this finding resides with the potential to utilize menthol pharmacokinetic (PK) data in experimental approaches which link PK and pharmacodynamic (PD) results in exploring the effect of menthol to treat pediatric patients with IBS or other disorders such as functional dyspepsia. For example, it is widely known that gastroparesis and delayed emptying can be seen in patients with IBS,

with gastrointestinal symptoms having an association with the degree of gastroparesis.[27] Use of a non-invasive technique such as ingestion of a small magnetic pill which is capable of providing objective information regarding motility of the entire gastrointestinal tract [28] could enable one to easily explore PK-PD relationships for menthol in patients with IBS. Such information will be critical in objectively determining both the effect and efficacy of menthol in the treatment of this disorder in children.

Recently, the European Medicines Agency has issued a public statement on the use of herbal medicinal products containing pulegone and menthofuran, two minor constituents (0.5 – 4.6% and 1 – 9%, respectively) of *M. piperita* oils.[29] Concerns regarding the potential associations of these compounds and hepatotoxicity in persons with a high daily intake of peppermint oil will likely spawn a renewed interest in this natural product, especially when it is used in a therapeutic context. The data from our pilot investigation of PMO have potential utility in the design of future studies designed to evaluate the dose-concentration-effect relationship for PMO and any compounds contained therein which may affect humans.

CONCLUSIONS

Our pilot study demonstrates that the PK of menthol, the active ingredient in PMO, can be adequately characterized in paediatric patients with IBS. It is important to recognize that formulation-specific differences between PMO-containing products can markedly influence the plasma menthol concentration vs. time curve. Such differences must be considered in the design of PK-PD studies of menthol in this disorder so as to enable an accurate characterization of the concentration-effect relationship.

ACKNOWLEDGEMENTS

We would like to thank Sabine Elkhoury and Alyssa Khan for their help coordinating the study and Ms. Judy Peat for performing the quantitation of menthol in patient specimens.

COMPETING INTERESTS STATEMENT

None of the co-authors have an existing business relationship with the manufacturer of the PMO formulation (Tillotts Pharma, Rheinfelden, Switzerland) used in the study nor do they have any other potential conflicts of interest to report that are related to the work described in this manuscript.

FUNDING STATEMENT

This study was supported in part by R01 NR013497(RJS) and K23 DK101688 (BPC) from the National Institutes of Health, the Daffy's Foundation (RJS), the USDA/ARS (RJS) under Cooperative Agreement No. 6250-51000-043, and P30 DK56338 which funds the Texas Medical Center Digestive Disease Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work is a publication of the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital. The contents do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

AUTHORS CONTRIBUTIONS

Dr. Gregory Kearns designed the pharmacokinetic portion of the study and assisted in the pharmacokinetic and statistical analysis of data. He also prepared the initial draft of the manuscript.

Dr. Bruno Pedro Chumpitazi contributed to the design of the clinical study protocol and provided oversight for the conduct of the clinical phases of the study. He contributed to the review and writing of the final version of the manuscript.

Dr. Susan M. Abdel-Rahman conducted the primary pharmacokinetic and biostatistical analysis of the study data. She contributed to the review and writing of the final version of the manuscript.

Dr. Uttam Garg was responsible for the development of the bioanalytical method used for the quantitation of menthol from plasma and provided oversight and quality control for the analysis of patient samples. He also contributed to the review and writing of the final version of the manuscript.

Dr. Robert J. Shulman contributed to the design of the clinical study protocol, its submission to the Institutional Review Board and to the final interpretation of the study data. As well, he also contributed to the review and writing of the final version of the manuscript.

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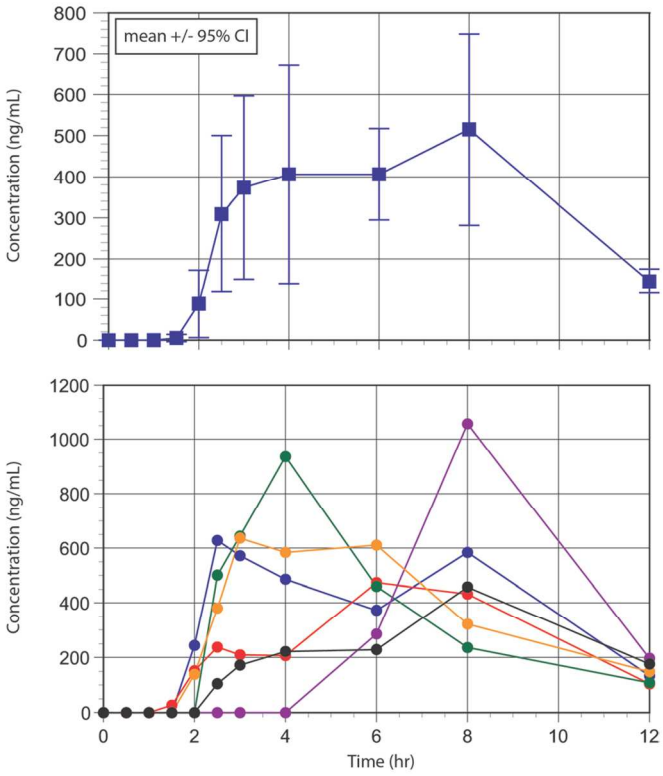

Mean (± 95% confidence interval) plasma concentration vs. time data for menthol in a cohort of six children with Irritable Bowel Syndrome given a single oral dose of peppermint oil containing approximately 83.0 mg of menthol [top panel] and individual plasma menthol concentration vs. time data [bottom panel]
215x279mm (150 x 150 DPI)

BMJ Open

Systemic Exposure to Menthol Following Administration of Peppermint Oil to Paediatric Patients

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2015-008375.R1
Article Type:	Research
Date Submitted by the Author:	15-Jun-2015
Complete List of Authors:	Kearns, Gregory; Children's Mercy Hospitals and Clinics, Research Development & Clinical Investigation Chumpitazi, Bruno; Baylor College of Medicine, Pediatrics Abdel-Rahman, Susan; Children's Mercy Hospitals and Clinics, Pediatric Pharmacology, Medical Toxicology & Therapeutic Innovation Garg, Uttam; Children's Mercy Hospitals and Clinics, Pathology & Laboratory Medicine Shulman, Robert; Baylor College of Medicine, Pediatrics
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	PAEDIATRICS, Paediatric gastroenterology < PAEDIATRICS, THERAPEUTICS

Systemic Exposure to Menthol Following Administration of Peppermint Oil to Paediatric Patients

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Key Words: Pharmacology, Therapeutics, Paediatrics

Word count: 3109

Figures: 1

Tables: 2

Disclosure / Conflict of Interest: None of the authors have any apparent conflict of interest related to the test agent used in this clinical trial and the company that provided it.

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ABSTRACT

Objective: Peppermint oil (PMO) has been used to treat abdominal ailments dating to ancient Egypt, Greece and Rome. Despite its increasing paediatric use as an IBS treatment, the PK of menthol in children given PMO has not been explored.

Design and Setting: Single-site, exploratory pilot study of menthol PK following a single 187 mg dose of PMO. Subjects with pediatric Rome II defined irritable bowel syndrome (IBS; n=6, male and female, 7-15 yr of age) were enrolled. Blood samples were obtained before PO administration and at 10 discrete time points over a 12 hr post-dose period. Menthol was quantitated from plasma using a validated GC-MS technique. Menthol pharmacokinetic (PK) parameters were determined using a standard non-compartmental approach.

Results: Following a dose of PMO, a substantial lag time (range 1-4 hr) was seen in all subjects for the appearance of menthol which in turn produced a delayed time of peak (Tmax = 5.3±2.4 hr) plasma concentration (Cmax = 698.2±245.4 ng/ml). Tmax and Tlag were significantly more variable than the two exposure parameters; Cmax, mean residence time (MRT) and total area under the curve (AUC=4039.7±583.8 ng/ml*hr) which had a coefficient of variation (CV) of <20%.

Conclusions: Delayed appearance of menthol in plasma after oral PMO administration in children is likely a formulation-specific event which, in IBS, could increase intestinal residence time of the active ingredient. Our data also demonstrate the feasibility of using menthol PK in children with IBS to support definitive studies of PMO dose-effect relationships. .

ARTICLE SUMMARY

Strength of the findings:

- An initial description of the concentration vs. time relationship for menthol following administration of a proprietary peppermint oil (PMO) formulation being used to treat paediatric patients with Irritable Bowel Syndrome.
- A basis for the design of future paediatric investigations of PMO designed to characterize the exposure-response relationship for menthol in children with Irritable Bowel Syndrome.

Limitations of the study:

- A small study cohort which, while providing descriptive informative regarding the clinical pharmacology of menthol from PMO in pediatric patients with IBS, may not reflect the true population variability in the dose vs. exposure relationship.

INTRODUCTION

Peppermint oil (Menthae piperitae aetheroleum) is obtained by steam distillation from the fresh leaves of peppermint (*Mentha piperita* L.). The major constituents of peppermint oil (PMO) include the terpenes (-) menthol (30-50%), (-) menthone (14-32%), (+) isomenthone (1.5-10%), (-) menthyl acetate (2.8-10%), (+) menthofuran (1.0-9.0%), and 1,8-cineol (3.5-14%). As reviewed by Grigoleit and Grigoleit [1], the pharmacologically active ingredient of PMO is menthol which in nature, exists as a pure stereoisomer (1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexanol).

Through its ability to act as a calcium antagonist, menthol appears to have a spasmolytic effect in the gastrointestinal tract. Consequently, PMO has been used to treat abdominal ailments dating to ancient Egypt, Greece and Rome [2] and anecdotal evidence of its purported efficacy abounds to this day. A review and several meta-analyses of randomized, double blind, placebo controlled trials in adults have demonstrated that PMO is effective in reducing abdominal pain in patients with irritable bowel syndrome (IBS). [1, 3-7] However, in children with IBS, there is a dearth of information restricted to a single small (n=42), double blind, placebo controlled trial. [7] Nonetheless, the use of PMO as an adjunctive measure to treat children with IBS continues to evolve in clinical practice despite the lack of any information pertaining to the impact of age and/or disease state on its pharmacodynamics or pharmacokinetics. Thus, dosing remains empiric in both paediatric patients and adults where a range in daily doses of more than 2.5-fold has been reported. [1]

Given the increasing use of PMO in pediatric patients with IBS and the potential for developmental changes to influence both the pharmacokinetics and pharmacodynamics of drugs [8], we conducted an exploratory, pilot pharmacokinetic (PK) study of menthol following the administration of a proprietary

formulation of PMO in a cohort of paediatric patients with IBS. The primary objective of this study was to examine the concentration vs. time profile of menthol following a single PMO dose and to describe its apparent PK .

METHODS

Patients

We performed a single-site, proof-of-principle exploratory study of menthol pharmacokinetics (PK) following a single 187 mg oral dose of PMO (Colpermin® capsule; Tillotts Pharma, Rheinfelden, Switzerland). Patients with paediatric Rome III defined IBS (n=6) who had no intercurrent illness or recent change in IBS symptoms were enrolled into a protocol approved by the Baylor College of Medicine Institutional Review Board. Children initially were identified by review of medical records. They were screened by telephone to be sure they qualified and that IBS symptoms were current. They were otherwise healthy. Absence of illness was assessed via history and physical examination at the time of the study visit. No participant was receiving therapeutic medications and/or natural products known to influence the activity of either hepatic drug metabolism (i.e., no enzyme inducers or inhibitors) or renal drug clearance. Finally, other than an existing diagnosis of IBS, no study participant had a history of an abnormality or surgery of the gastrointestinal tract.

All study participants were enrolled by informed parental consent and when appropriate (e.g. > 6 years of age), by patient assent. Informed consent was documented prior to performing any study-related procedure.

Drug administration, sampling and sample handling

The clinical phase of this study was conducted in strict accordance with Good Clinical Practice principles. Prior to being admitted to the Metabolic Research Unit (MRU) at the Children's Nutrition Research Center, study participants were fasted from midnight immediately preceding the morning of admission (i.e., overnight). Following the history, physical examination and recording of vital signs (temperature, pulse rate, respiratory rate, seated blood pressure), a venous cannula (21 gauge) was aseptically placed in a large vein either in the dorsum of the hand or on the volar surface of the lower arm to facilitate obtaining repeated blood samples to support the PK objectives of the study. The cannula was secured and its patency maintained through periodic flushing of the dead space with sterile 0.9% sodium chloride.

At approximately 0900, subjects received a single oral dose of PMO given as a commercially available, proprietary, non-prescription product (Colpermin®, each capsule containing approximately 83.0 mg of menthol as a constituent of PMO) followed by 120 mL of water at 25°C. Immediately prior to administration of the test article, a blood sample (2.0 mL) was obtained. After PMO administration, repeated blood samples (2.0 mL each) were obtained directly into green top glass tubes containing sodium heparin (Vacutainer®, Becton Dickinson, East Rutherford, NJ) at the following post-dose time points: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours. At 2 hours after PMO administration, participants were given a standardized meal. Throughout the 12-hour study period, participants were restricted from any strenuous physical exercise. After completion of the final (12-hour) sample, vital signs were reassessed, the venous cannula was removed and the insertion site evaluated for redness, swelling and/or bruising. Study participants were then discharged from the MRU and received a follow up call

from a clinical research coordinator approximately 24 hours thereafter to specifically assess/evaluate any potential adverse effects.

Immediately following collection of a given blood sample, it was mixed by gentle inversion and the tube immediately placed at 4°C. At 4-hour intervals, blood samples were centrifuged (2,500 x g for 10 minutes at 4°C). The resultant plasma from each tube then was immediately transferred into a clean, screw capped polypropylene tube and immediately frozen at -80°C. Samples were maintained at 2-8°C while handling so as to minimize evaporative loss of any volatile compounds contained therein.

Analytical

Total (i.e., conjugated and un-conjugated) menthol in plasma was measured by gas chromatography mass spectrometry (GC-MS) as described in previous methods with several minor modifications.[9, 10] Briefly, to 0.5 mL of the patient's heparinized plasma or controls or calibrators, 20 µL internal standard (menthol-d4, 10 µg/mL), 25 µL β-glucuronidase (90,000 U/mL), and 10 µL sodium acetate buffer (pH 4.8) were added. The mixture was incubated overnight in air tight tubes in 37^{0}C water bath and to each tube, 150 µL 0.4 M phosphate buffer (pH 4.8) was added. Menthol was extracted in 0.5 mL methylene chloride by rocking the tubes for 5 minutes. The mixture was centrifuged at 2000 g for 5 minutes. The methylene chloride layer was transferred to an auto-sampler vial and a 2 µL extract was injected onto the GC-MS (Agilent Technologies, Santa Clara, CA) installed with ZB-1MS 15m x 250um x 0.25 µm column. Ions (m/z) monitored for quantification and identification were 138, 123, 95 for menthol and 142, 127, 99 for menthol-d4. The data were analyzed using Target Software (Thru-Put Systems, Orlando, FL). The quantitative ions were used to construct standard curves of the peak area ratios (calibrator/internal standard pair). The assay was linear within the range of concentrations

evaluated 5-1000 ng/mL (r^2>0.99). For all standards the intra- and inter-assay coefficient of variations (CVs) were <10%.

Pharmacokinetic data analysis

Menthol plasma concentration versus time data were evaluated using a model independent approach. Individual Cmax and Tmax were obtained by direct examination of the plasma concentration versus time profile. The area under the plasma concentration versus time curve during the sampling period (AUC_{0-n}) was calculated using the mixed log-linear method where "n" refers to the final sampling time with quantifiable menthol concentrations. The terminal elimination rate constant (λz) was estimated using an iterative least-squares regression algorithm when a sufficient number of post-peak concentrations were available to support a reliable estimation of the parameter. When possible, the total AUC (AUC_{tot}) was extrapolated to infinity by dividing λz into the predicted plasma concentration at the end of the sampling interval. As a subset of our patients (n=3) did not have > 3 post-Cmax plasma concentrations, there was significant uncertainty associated with the estimation of λz and consequently the pharmacokinetic parameters that rely on the terminal rate constant [e.g. AUC to infinity ($AUC_{0-\infty}$), apparent oral clearance (Cl/F), apparent volume of distribution at steady-state (Vss/F)] are not reported. Menthol pharmacokinetic data were examined using standard descriptive statistics. *A priori* determination of statistical power was not considered given that this was the first PK study performed in a pediatric cohort, the goal of this exploratory study was descriptive and no comparisons (i.e., within- or between group) of PK data were undertaken. All analyses were performed in Kinetica™ version 5.1 (Thermo Scientific) and SPSS version 20 (IBM SPSS).

RESULTS

All six participants completed the study without experiencing any apparent adverse events. The study cohort consisted of 4 female and 2 male patients. Their age range was 7 to 12 years (10.3±1.9 yr.) and their body weights ranged from 26.6 to 58.2 kg (45.2±11.5 kg). None of the participants had a height or weight which was outside of the 5th to 95th percentile for age.

The composite (mean±95% confidence limits) and individual plasma menthol concentration vs. time data are shown in Panels A and B of the Figure, respectively. Considerable intersubject variability in the plasma concentration vs. time profiles was apparent with all participants in the cohort. Individual pharmacokinetic (PK) parameters for menthol are provided in Table 1. The time of appearance (Tmax) for apparent peak plasma concentrations (Cmax) ranged from 2.5 to 8 hours following a lag time (Tlag) which ranged from 1.5 to 4 hr. Absolute Cmax values ranged from 458 to 1056 ng/mL which when corrected to a weight adjusted menthol dose received by each child, ranged from 255 to 470 ng/mL per 1 mg/kg (i.e., an approximate 1.8-fold difference). The dose normalized systemic menthol exposure reflected by the area under the plasma concentration vs. time curve (AUC per mg/kg dose) varied over an approximate 2-fold range (1464.1 to 2941.1 ng/mL*hr). Given the marked delay in the appearance of menthol in plasma after a PMO dose, sufficient post-peak plasma concentrations to reliably estimate an apparent elimination rate constant (λz) and elimination half-life were not available in all participants. Consequently, the non-compartmental parameter mean residence time (MRT; a time representing elimination of approximately 60% of a given dose from the body) was determined using statistical moment theory and ranged from 6.4 to 9.4 hours (Table 1).

Table 1: Individual Menthol Pharmacokinetic Parameters

Participant	Menthol dose (mg/kg)	Cmax (ng/mL)	Cmax (ng/mL per mg/kg dose)	Tmax (h)	Tlag (h)	AUClast (ng/mL*h per mg/kg dose)	AUCtot (ng/mL*h per mg/kg dose)	MRT (h)
MentPK-01	1.4	628	445.1	2.5	1.5	2941.1	3199.2	7.0
MentPK-02	1.5	474	325.0	6	1	2045.6	2245.9	7.5
MentPK 03	2.0	936	470.5	4	2	1951.4	2137.1	6.4
MentPK 04	3.1	1056	342.1	8	4	1198.4	1350.5	9.1
MentPK 05	1.9	637	332.8	3	1.5	2107.2	2434.8	7.4
MentPK 06	1.8	458	255.5	8	2	1464.1	1879.5	9.4

Abbreviations include: Cmax, apparent peak plasma concentration; Tmax, time of Cmax; Tlag, apparent lag time between PO administration and appearance of menthol in plasma; AUC, area under the plasma concentration vs. time curve and MRT, mean residence time.

A summary of the pharmacokinetic parameters (mean, SD and 95% confidence intervals) is provided in Table 2. As reflected by the values for the coefficient of variation for each of the parameters, the least variability was observed for MRT (CV = 15.4%) and AUCtot (14.4%).

Table 2: Summary of Menthol Pharmacokinetic Parameters

Parameter	mean ± SD	95% CI	CV%
Cmax (ng/mL)	698.2 ± 245.4	[501.8, 894.6]	35.1
Cmax (ng/mL per mg/kg)	361.8 ± 80.8	[297.2, 426.5]	22.3
Tmax (h)	5.3 ± 2.4	[3.3, 7.2]	45.3
Tlag (h)	2.0 ± 1.0	[1.2, 2.8]	50.0
AUClast (ng/mL*h)	3562.0 ± 616.7	[3068.5, 4055.4]	17.3
AUC last (ng/mL*h per mg/kg dose)	1951.3 ± 602.8	[1468.9, 2433.7]	30.9
AUCtot (ng/mL*h)	4039.7 ± 583.8	[3572.6, 4506.9]	14.4
AUC total (ng/mL*h per mg/kg dose)	2207.8 ± 613.8	[1716.7, 2698.9]	27.8
%AUCextrapolated	12.1 ± 5.3	[7.8, 16.3]	43.8
MRT (h)	7.8 ± 1.2	[6.8, 8.8]	15.4

Abbreviations: SD, standard deviation; CI, confidence interval; CV, coefficient of variation. Pharmacokinetic parameter abbreviations contained in footnote of Table 1

DISCUSSION

The putative active ingredient contained in PMO is menthol, a plant-derived, semi-volatile monoterpene. The clinical pharmacology of menthol, with an emphasis on its interaction with sensory neurons (TRP channels), has been recently reviewed. [11] The disposition and pharmacokinetics of menthol derived from the ingestion of peppermint oil in adults also has been reported previously [12-14] as have the pharmacokinetics of *L*-menthol administered directly to the upper gastrointestinal tract. [15] In a cohort of 12 adults administered an oral dose of *L*-menthol (10 and 100 mg), the drug was

rapidly metabolized with conversion to a menthol glucuronide which could be measured in plasma and urine.[14] In a similar study of 16 adults reported by Mascher, et al. [13], the apparent time of peak plasma concentration (Tmax) of menthol (measured by GC/MS) following oral administration of a 180 mg dose of enteric coated PMO occurred at approximately 3 hours following drug administration. The decline of methanol levels in the plasma was rapid with an average apparent elimination half life of approximately 3.5 hours.

Recent studies have shown that menthol clearance from plasma is associated with the activity of CYP2A6 [16], a polymorphically expressed cytochrome P450 isoform that is responsible for the biotransformation of other xenobiotics such as the primary nicotine metabolite, cotinine [17] and the commonly used antimicrobial agent, metronidazole.[18] In the case of cotinine, Dempsey, et al. [17] demonstrated that *CYP2A6* genotype (a surrogate for CYP2A6 phenotype), as opposed to age, was the major determinant of cotinine plasma elimination half-life in infants and children. To date, the PK of menthol have not been evaluated or reported in a pediatric patient cohort. Thus, there are no data upon which to base the development of PMO dosing regimens for children or adolescents with IBS to produce desired levels of systemic menthol exposure.

Despite data from only a single available pediatric trial of PMO in children with IBS [7], the substance is being increasingly used either as adjunctive or primary treatment of patients with this disorder based on anecdotal evidence of symptomatic improvement. It is not known whether the potential analgesic effects of menthol in patients with IBS have a central [19] and/or local basis. In contrast, the antispasmodic effects appear to be modulated locally as demonstrated by Hiki, et al. [15] in a study where *L*-menthol was directly sprayed onto gastric mucosa. It is for this reason that the Colpermin®

formulation of PO is an enteric-coated solid oral dosage form designed to release the active ingredients at a pH encountered in the small intestine (i.e.,> 6.5). [20]

As illustrated in the Figure, the appearance of menthol in the plasma of all subjects in our cohort was delayed with an average lag time of 2 hours; a finding consistent with aggregate data in adults summarized by the manufacturer of our test article. [20] This finding is supported to some extent by two earlier studies of the Colpermin® product which examined the pharmacokinetics of menthol in adults using urinary menthol excretion data [21,22] and a more recent adult study which examined menthol concentrations in plasma following the administration of a different enteric-coated PMO product (Enteroplant®, Spitzner Pharmaceuticals, Ettlingen, Germany). [23] Unlike the plasma concentration profile reported by Mascher, et al. [23], the mean plasma menthol concentration vs. time data in our paediatric study cohort (Figure; top panel) demonstrated an apparent prolonged absorption time with apparent peak plasma concentrations (Tmax) occurring between 2.5 and 8 hours post-dose (Table 1). Reasons for the delayed Tmax likely reside with formulation-specific factors (i.e., delayed release) and potentially, enterohepatic recirculation, as menthol undergoes biotransformation by UDP-glucuronosyltransferase 2B7 (UGT2B7).[24, 25] The apparent post-Tmax increase in plasma methanol concentrations in several of our study participants (Figure, bottom panel) suggests the presence of enterohepatic recirculation.

In contrast to the adult data reported by Masher, et al. [23], we were not able to reliably estimate the apparent terminal elimination rate constant (λz) for menthol in our entire patient cohort; namely, patients 2, 4 and 6 where ≤ 2 post-Cmax plasma concentrations were available for estimation of the parameter. It was for this reason that we chose to use a non-compartmental approach to describe menthol pharmacokinetics and to examine mean residence time (MRT) as a parameter reflecting drug

elimination. As noted above, our inability to produce a reliable estimate of λz precluded our reporting reliable estimates for the apparent plasma clearance and/or volume of distribution for menthol. Also, given the prolonged absorption for menthol in our cohort (Figure), estimation of λz may have produced an errant result consequent to the phenomenon of the "flip-flop model" which occurs when the rate of absorption and/or distribution is slower than the rate of true drug elimination.[26] Nonetheless, based upon known patterns of ontogeny with regard to the activity of UGT isoforms [8], we would not expect that the apparent elimination half life of menthol in our study participants (i.e., range from 1.6 to 3.0 hr in 3 patients with reliable λz estimates) would be similar to values for this parameter (3.5 to 4.4 hours) previously reported from adults. [23] A more accurate estimate of λz would have been available in our cohort had we previously had knowledge of the prolonged menthol absorption profile. This would have enabled us revise our blood sampling paradigm to cluster observations between 6 and 18 hours post-dose and to extend the sampling interval to 24 hours.

Despite considerable variability in the plasma menthol concentration vs. time data in our patients (Figure), the values for the AUCtot and MRT had the smallest coefficients of variation associated with them (Table 2). This relatively close agreement in the PK parameters suggested by the coefficients of variation would seemingly support that despite the presence of allelic variants for *CYP2A6* [17] and *UGT2B7* [25] in the general population, the concentration vs. time data for menthol in our cohort did not appear to contain an "outlier" which could have been attributable to one or more of the participants having inherited an allelic variant for either of these genes.

Admittedly, the results from our exploratory study are descriptive and given the small size of the study cohort, may not be generalizable to a larger population of pediatric patients with IBS. The potential value of our findings resides with the provision of preliminary PK data for menthol in pediatric patients

that could be used to inform and enrich the design of future trials to explore the link between menthol PK and pharmacodynamics (PD) in patients with IBS or other disorders such as functional dyspepsia. For example, it is widely known that gastroparesis and delayed emptying can be seen in patients with IBS, with gastrointestinal symptoms having an association with the degree of gastroparesis. [27] Use of a non-invasive technique such as ingestion of a small magnetic pill which is capable of providing objective information regarding motility of the entire gastrointestinal tract [28] could enable one to easily explore PK-PD relationships for menthol in patients with IBS. Such information will be critical in objectively determining both the effect and efficacy of menthol in the treatment of this disorder in children and adolescents.

Recently, the European Medicines Agency has issued a public statement on the use of herbal medicinal products containing pulegone and menthofuran, two minor constituents (0.5 – 4.6% and 1 – 9%, respectively) of *M. piperita* oils. [29] Concerns regarding the potential associations of these compounds and hepatotoxicity in persons with a high daily intake of peppermint oil will likely spawn a renewed interest in this natural product, especially when it is used in a therapeutic context. While the quantitation of pulegone or menthofuran from the plasma samples of our study participants or the test article was beyond the scope of our study, the human health consequences of their presence as "contaminants" of commercially available PMO formulations are not yet known. Nonetheless, the data from our exploratory study of PMO may have utility in the design of future studies designed to evaluate the dose-concentration-effect relationship for PMO and any compounds contained therein.

CONCLUSIONS

Our pilot study demonstrates that the PK of menthol, the active ingredient in PMO, can be adequately characterized in paediatric patients with IBS. It is important to recognize that formulation-specific differences between PMO-containing products can markedly influence the plasma menthol concentration vs. time curve. Such differences must be considered in the design of PK-PD studies of menthol in this disorder so as to enable an accurate characterization of the concentration-effect relationship.

ACKNOWLEDGEMENTS

We would like to thank Sabine Elkhoury and Alyssa Khan for their help coordinating the study and Ms. Judy Peat for performing the quantitation of menthol in patient specimens.

COMPETING INTERESTS STATEMENT

None of the co-authors have an existing business relationship with the manufacturer of the PMO formulation (Tillotts Pharma, Rheinfelden, Switzerland) used in the study nor do they have any other potential conflicts of interest to report that are related to the work described in this manuscript.

DATA SHARING STATEMENT

No additional date available.

FUNDING STATEMENT

This study was supported in part by the Daffy's Foundation (RJS), the USDA/ARS (RJS) under Cooperative Agreement No. 6250-51000-043, and P30 DK56338 which funds the Texas Medical Center Digestive Disease Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work is a publication of the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital. The contents do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

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AUC last (ng/mL*h per mg/kg dose)	1951.3 ± 602.8	[1468.9, 2433.7]	30.9
AUCtot (ng/mL*h)	4039.7 ± 583.8	[3572.6, 4506.9]	14.4
AUC total (ng/mL*h per mg/kg dose)	2207.8 ± 613.8	[1716.7, 2698.9]	27.8
%AUCextrapolated	12.1 ± 5.3	[7.8, 16.3]	43.8
MRT (h)	7.8 ± 1.2	[6.8, 8.8]	15.4

Abbreviations: SD, standard deviation; CI, confidence interval; CV, coefficient of variation. Pharmacokinetic parameter abbreviations contained in footnote of Table 1

Figure Legend

Mean (± 95% confidence interval) plasma concentration vs. time data for menthol in a cohort of six children with Irritable Bowel Syndrome given a single oral dose of peppermint oil containing approximately 83.0 mg of menthol [top panel] and individual plasma menthol concentration vs. time data [bottom panel]

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AUTHORS CONTRIBUTIONS

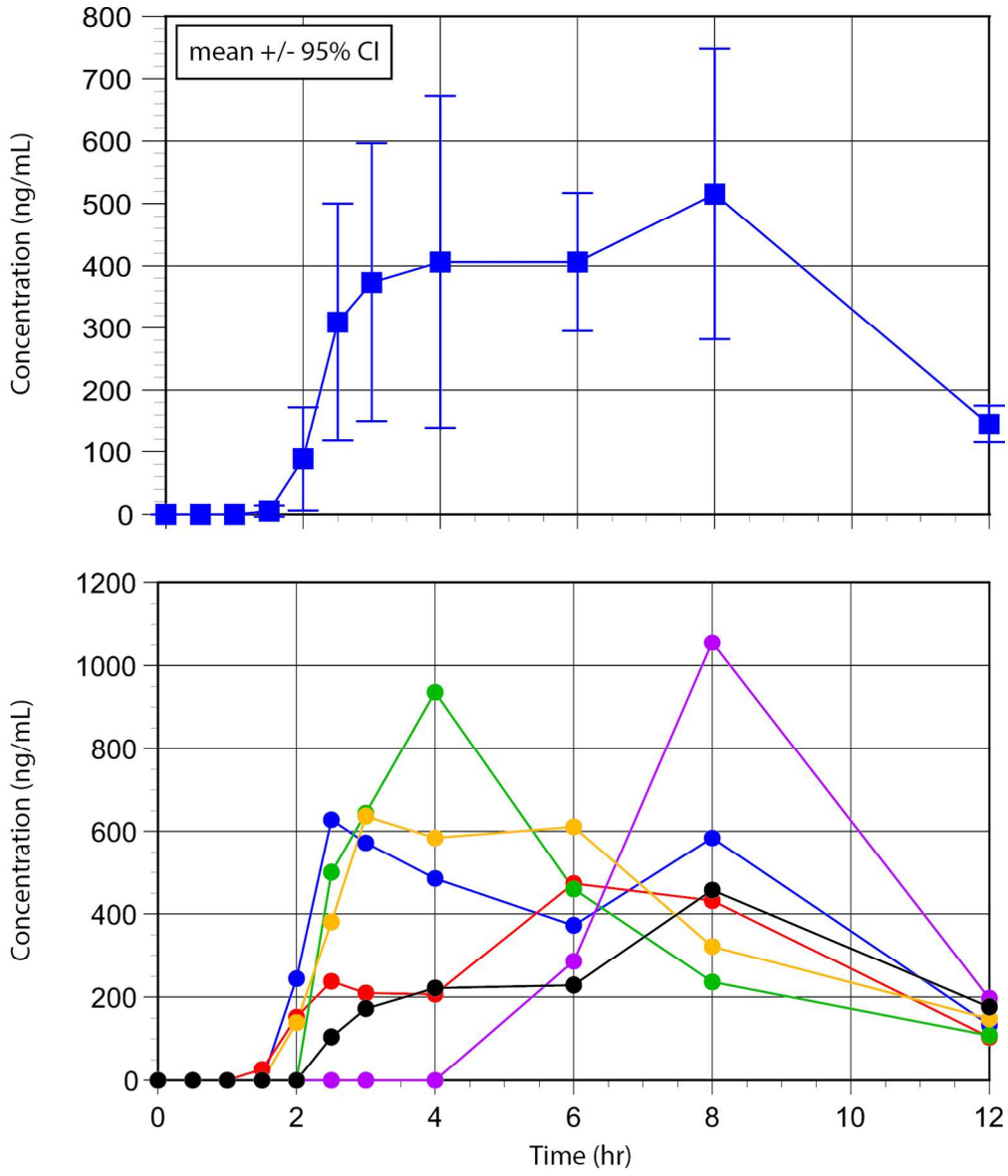
Dr. Gregory Kearns designed the pharmacokinetic portion of the study and assisted in the pharmacokinetic and statistical analysis of data. He also prepared the initial draft of the manuscript.

Dr. Bruno Pedro Chumpitazi contributed to the design of the clinical study protocol and its submission to the Institutional Review Board. He contributed to the review and writing of the final version of the manuscript.

Dr. Susan M. Abdel-Rahman conducted the primary pharmacokinetic and biostatistical analysis of the study data. She contributed to the review and writing of the final version of the manuscript.

Dr. Uttam Garg was responsible for the development of the bioanalytical method used for the quantitation of menthol from plasma and provided oversight and quality control for the analysis of patient samples. He also contributed to the review and writing of the final version of the manuscript.

Dr. Robert J. Shulman contributed to the design of the clinical study protocol, provided oversight for the conduct of the clinical phases of the study, and to the final interpretation of the study data. As well, he also contributed to the review and writing of the final version of the manuscript.



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Mean (± 95% confidence interval) plasma concentration vs. time data for menthol in a cohort of six children with Irritable Bowel Syndrome given a single oral dose of peppermint oil containing approximately 83.0 mg of menthol [top panel] and individual plasma menthol concentration vs. time data [bottom panel]

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