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Pharmacogenetic Trial of a Cannabinoid Agonist Shows Reduced Fasting Colonic Motility in Patients with Non-Constipated Irritable Bowel Syndrome

Banny S. Wong, M.D.^{*}, Michael Camilleri, M.D.^{*}, Irene Busciglio, B.S.^{*}, Paula Carlson, B.S.^{*}, Lawrence A. Szarka, M.D.^{*}, Duane Burton^{*}, and Alan R. Zinsmeister, Ph.D.[†]

^{*}Clinical Enteric Neuroscience Translational and Epidemiological Research (CENTER), Division of Biomedical Statistics and Informatics College of Medicine, Mayo Clinic Rochester, Minnesota

[†]Department of Health Sciences Research, Division of Biomedical Statistics and Informatics College of Medicine, Mayo Clinic Rochester, Minnesota

Abstract

Background—Cannabinoid receptors are located on cholinergic neurons. Genetic variants that affect endocannabinoid metabolism are associated with colonic transit in patients with irritable bowel syndrome with diarrhea (IBS-D). We compared the effects of dronabinol, a non-selective agonist of the cannabinoid receptor, with those of placebo on colonic motility and sensation in patients with IBS, and examined the effects of IBS subtype and specific genetic variants in cannabinoid mechanisms.

Methods—Seventy-five individuals with IBS (35 with IBS with constipation [IBS-C], 35 with IBS-D, and with 5 IBS-alternating [IBS-A]) were randomly assigned to groups that were given 1 dose of placebo or 2.5 mg or 5.0 mg dronabinol. We assessed left colonic compliance, the motility index (MI), tone, and sensation, during fasting and after a meal. We analyzed the single nucleotide polymorphisms *CNR1 rs806378*, *FAAH rs324420*, and *MGLL rs11538700*.

Results—In all patients, dronabinol decreased fasting proximal left colonic MI, compared with placebo (overall $P=.05$; for 5 mg dronabinol, $P=.046$), decreased fasting distal left colonic MI (overall $P=.08$; for 5 mg, $P=.13$), and increased colonic compliance ($P=.058$). The effects of dronabinol were greatest in patients with IBS-D or -A (proximal colonic MI, overall $P=.022$; compliance, overall, $P=.03$). Dronabinol did not alter sensation or tone. *CNR1 rs806378* (CC vs CT/TT) appeared to affect fasting proximal MI in all patients with IBS ($P=.075$). Dronabinol affected fasting distal MI in patients, regardless of *FAAH rs324420* variant (CA/AA vs CC) ($P=.046$); the greatest effects were observed among IBS-C patients with the *FAAH* CC variant ($P=.046$).

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Corresponding author: Michael Camilleri, M.D. Mayo Clinic Charlton 8-110 200 First Street S.W. Rochester, MN 55905
camilleri.michael@mayo.edu Phone: 507-266-2305.

Authors' contributions: M. Camilleri: study conceptualization, endoscopic placements, writing of protocol and manuscript; B. Wong: research fellow, recruitment, assisting with measurements, writing protocol and manuscript; L.A. Szarka: endoscopic placements; A.R. Zinsmeister: database management and statistical analysis; I. Busciglio: participant recruitment, sensation and motility studies; D. Burton: endoscopic placements; P. Carlson: genotyping

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045). Dronabinol affected fasting proximal MI in patients with IBS-D or -A with the variant *FAAH* CA/AA ($P=.013$).

Conclusion—In patients with IBS-D or -A, dronabinol reduces fasting colonic motility; *FAAH* and *CNR1* variants could influence the effects of this drug on colonic motility.

Keywords

sensory; motor; clinical trial; drug metabolism; pharmacodynamic

INTRODUCTION

The effects of cannabinoids are mediated primarily through cannabinoid receptors. Two types of G protein coupled cannabinoid receptors, CB₁ and CB₂, have been identified and cloned^{1–3}. There may be a third, as yet uncloned, cannabinoid receptor⁴. CB₁-immunoreactivity is located on normal colonic epithelium, smooth muscle, and the myenteric plexus, whereas both CB₁ and CB₂ receptors are expressed in plasma cells⁵. The endocannabinoid system consists of CB₁ and CB₂ receptors; the ligands of these receptors are anandamide and 2-arachidonyl glycerol (2-AG), and the ligand-inactivating enzymes are monoacylglycerol lipase (MGLL) and fatty acid amide hydrolase (FAAH)^{6–9}.

The activity of the endocannabinoid system varies between species and in different regions of the gastrointestinal (GI) tract within the same species. On the other hand, activation of CB₁ receptors coupled to cholinergic motor neurons inhibits excitatory nerve transmission in human colonic circular muscle¹⁰ in vitro. In mice, endocannabinoids acting on myenteric CB₁ receptors tonically inhibit colonic propulsion¹¹. In rodent models, activation of enteric cannabinoid CB₁ receptors inhibits gastric and small intestinal transit without altering intraluminal pressure or basal tone^{12,13}. In a prior study in healthy volunteers, we have shown that dronabinol, a non-selective CB receptor agonist, inhibits gastric emptying and colonic motility in healthy humans^{14,15}. The effects on colonic tone and phasic motility were observed with 7.5mg dronabinol, which was shown to induce drowsiness, lightheadedness and dizziness¹⁵. The 5mg dronabinol dose was more tolerable among healthy participants in our previous study. CB receptors are also involved in mediating nociception^{16,17} and inflammation¹⁸.

In this study, we assessed the effects of 5mg dronabinol on colonic sensory and motor functions in patients with irritable bowel syndrome (IBS) who were cannabinoid-naïve. We hypothesized that dronabinol inhibits colonic motility and sensation in IBS, and that these inhibitory effects are affected by genetic variations in the CB₁ receptor and in rate-limiting enzymes of endocannabinoid degradation.

Our specific aims were: 1) to compare effects of single administrations of oral placebo, dronabinol 2.5mg, and dronabinol 5mg on colonic motility and sensation in cannabinoid-naïve IBS patients; and 2) to examine potential influences of IBS subtypes and genetic variations in cannabinoid mechanisms on the effects of dronabinol treatment. In the latter aim, we examined effects of variations in critical genes for CB signaling (CB₁ type1), genes involved in metabolic breakdown of anandamide and 2-acylglycerol, FAAH and MGLL respectively, and *CYP2C9**3 which significantly alters the metabolism of dronabinol on colonic motor and sensory functions observed in response to treatment with dronabinol in IBS patients.

MATERIALS AND METHODS

Study Design

This was a double-blind, randomized, placebo-controlled, parallel-group study (ClinicalTrials.gov identifier NCT01253408) of the pharmacodynamic effects of dronabinol on colonic sensory and motor functions of otherwise healthy human volunteer participants with IBS (ages between 18 and 67 years, and body mass index between 18 and 47 kg/m²). The study was conducted in the Clinical Research Unit at Mayo Clinic in Rochester, MN (NIH CTSA grant RR0024150); the study started October 2008 and was completed November 2010. The study was approved by Mayo Clinic Institutional Review Board, and a data safety monitoring plan was established prior to starting the study.

Participants

All participants were recruited from a database of ~1000 patients with IBS who reside within 120 miles of Rochester, MN. Participants filled in a validated bowel disease questionnaire (BDQ, including questions to correspond to Rome III criteria)¹⁹ and the Hospital Anxiety and Depression Inventory (HAD)²⁰. The bowel disease questionnaire also included a somatic symptom checklist intended to identify somatization. All candidates were screened to ensure they were cannabinoid-naïve, and those who met the eligibility criteria for the study underwent a complete history and physical examination before enrollment. The trial flow is summarized in Figure 1.

Ultimately, 75 otherwise healthy participants with IBS were enrolled, and 72 completed most studies characterizing the intermediate phenotypes. All females of childbearing potential had to have a negative pregnancy test within 48 hours of study. Participants were randomized to one oral administration of placebo, dronabinol 2.5mg, or dronabinol 5mg, taken with water at the study center under supervision of study staff.

Randomization with 24 per treatment group was conducted by computer program. Allocation was concealed, and participants and investigators were blinded to all treatment assignments. The research pharmacist ensured the random allocation sequence was followed and that participants were assigned to the appropriate group. At study completion, the randomization code was communicated to the study statistician by the research pharmacist.

Pharmacology of Dronabinol

Dronabinol is a synthetic delta-9-tetrahydrocannabinol (Δ^9 -THC). It is a non-selective cannabinoid agonist with affinity for both CB₁ and CB₂ receptors; 90–95% of the dose is absorbed after a single oral dose²¹. Due to the high first pass hepatic metabolism (primarily by microsomal hydroxylation) and lipid solubility, only 10–20% of the administered oral dose reaches the systemic circulation. The onset of action after oral administration is at 0.5 to 1 hour, and the peak effect is at 2 to 4 hours. The elimination phase follows a two-compartment model with an initial half-life of ~4 hours and a terminal half-life of 25–36 hours. Biliary excretion is the major route of elimination.

Experimental Protocol

After overnight bowel preparation using a standard polyethylene glycol-containing electrolyte solution (GoLyte, Braintree Laboratories, Inc., Braintree, MA) and a 12-hour fast to induce cleansing, a balloon-manometry assembly was placed in the mid-descending or upper sigmoid colon of each participant with the aid of unsedated left-side colonoscopy, guidewire placement and fluoroscopy. Details of the catheter, barostat, and conduct of sensation and motility testing are provided in the Appendix, and followed the procedures in prior studies^{22,23}.

The study medication was ingested, and 1 hour later the same colonic functions were assessed in the fasting state: the 30 minute post-drug tone first, followed by a second VAS scale to assess the levels of tension, relaxation, energy and drowsiness, and then the compliance and randomly ordered phasic distensions as previously done pre-drug. Subsequently, colonic tone and phasic pressure activity were measured for 30 minutes before and 1 hour after a standard 1000kcal liquid meal (750mL chocolate milkshake, 53% fat, 35% carbohydrate, 12% protein). When the recording was finished, the balloon was deflated and the tube was removed by gentle traction.

Data Analysis and Outcome Measures

Colonic compliance—We used a validated linear interpolation method to estimate compliance of the colon, summarized as the pressure at half-maximum volume (PR₅₀ or PR₅₀)²⁴.

Colonic motor function—Colonic tone was assessed operationally as the intracolonic balloon volume measured at the operating pressure. Tone was calculated by the baseline colonic volumes measured with the same intra-balloon operating pressure throughout the period of interest during fasting (before and after drug) or after the meal^{25,26}. Using computer-based 10-minute mean volumes for the periods of interest and using the mean of each 10-minute observation in those periods, changes in colonic tone were calculated as absolute volume changes during fasting in response to the study medication and as the symmetric percent change in volume postprandially²³.

Colonic manometry—The same computer program was used to measure the postprandial phasic motor activity in the proximal and distal three manometric sensors. Because of variation in the location of the barostat balloon in the upper or lower descending colon, the phasic activity was summarized in each individual for the 3 sensors that were located in the distal descending and sigmoid colon. Data for the fasting period were compared with the two 30-minute periods after the 1000kcal meal was ingested. Colonic phasic pressure activity was summarized as a motility index (MI) where: $MI = \log_e (\text{sum of amplitudes} * \text{number of contractions} + 1)$

Colonic sensation—We recorded the pressure thresholds at which participants reported first perception, gas, and pain during the assessment of colonic compliance (ramp distention), and the intensity ratings recorded for gas and pain on 100mm VAS scales that were averaged over the 4 phasic pressure distensions (termed the mean sensation rating). We assessed stress and arousal scores while we assessed the effects of treatment on sensation. As there were no significant effects noted based on the scores of tension, relaxation, energy and drowsiness, the sensation results are provided without adjusting for these measurements.

Genotyping

DNA was extracted from whole blood as previously described²⁷. The selection of candidate endocannabinoid genetic polymorphisms is included in the Appendix. Genotyping of *FAAH* rs324420, *CNR1* rs806378, and *MGLL* rs4881 was performed using Taqman™ SNP Genotyping assays (Applied Biosystems, Inc., Foster City, CA) in accordance with manufacturer instructions. In addition, we screened patients for the *CYP2C9* rs1057910 polymorphism (A1075C; Ile359Leu), also known as *CYP2C9**3, since this variant significantly alters the metabolism of orally administered dronabinol²⁸, with a three-fold increase in plasma dronabinol levels in CC homozygotes, but only a modest increase in heterozygotes compared to “wildtype” AA homozygotes.

Sample Size Assessment

Appendix Table 1 shows the coefficients of variation and effect sizes demonstrable with $n=24$ per group, based on pre-treatment data or post-treatment placebo group data of motor and sensory endpoints for the participants in this study, using 80% power with a two-sided α of 0.05 in a two-sample t-test.

Statistical Analysis

The study statistician and the entire research team were blinded to treatment allocation until all analyses of motor and sensory endpoints had been completed. All subjects randomized were included in the analysis under the intention to treat (ITT) paradigm. Subjects with missing data had the corresponding values imputed using the overall subjects mean (or median). An analysis of covariance (ANCOVA) was used to assess treatment effects on colonic tone, compliance and VAS sensation rating scores, incorporating gender, BMI and the corresponding “baseline” or pre-drug value as covariates. An adjustment in the error degrees of freedom was made (subtracting one for each missing value imputed) to adjust the estimates of error variance in the ANCOVA models. In the overall analyses of treatment effects (i.e. ignoring IBS subgroup and genotype) and in the assessment of potential differential treatment effects among IBS subgroups, 4–7 missing values were imputed, depending on the particular quantitative trait or endpoint assessed (e.g. distal MI was missing in 7, and PR₅₀ was missing in 4). In the pharmacogenetic analyses, 73 (*CNRI*), 71(*FAAH*), and 72(*MGLL*) of the 75 subjects had genotype status identified. Among the genotyped subjects, 1 to 4 missing values for the intermediate phenotype endpoints were imputed.

For the analysis of VAS sensation scores, analyses of the scores at the 40mmHg distension level and, separately, for the corresponding post-drug average (over all 4 distensions) were examined. The overall average baseline sensory rating score during the pre-drug study was the “corresponding” baseline value used as a covariate in these analyses.

A proportional hazards regression analysis was used to assess treatment effects on sensation thresholds, incorporating gender, BMI, and the corresponding pre-treatment sensory threshold value as covariates.

The analyses were repeated including IBS subgroup (combining IBS-D and IBS-A, since the latter have been shown to have accelerated transit at 48hours, similar to IBS-D)²⁹ and, separately, the *CNRI*, *FAAH* and *MGLL* genotypes (dominant genetic model grouping the minor allele homozygotes together with heterozygotes) as covariates, along with the corresponding treatment by subgroup interaction terms. Due to the small minor allele frequency (MAF) of the *MGLL* rs4881 SNP, for both the *CNRI* rs806378 and *FAAH* rs324420 SNPs we assessed potential differential drug vs. placebo treatment effects by combining the 2.5 and 5mg dronabinol doses. Finally exploratory analyses incorporating IBS subtype and each genotype subtype (separately) were also examined to check for potential differential treatment effects by IBS subtypes and candidate genotypes.

RESULTS

Participants and Compliance with Medication

The trial flow is shown in Figure 1. Seventy-five IBS volunteers meeting the entry criteria were screened and randomized, with a total of 72 completing the study. A total of 27 volunteers randomly received placebo, 24 received dronabinol 2.5mg, and 24 received dronabinol 5mg. The table in Figure 1 summarizes patient demographics by treatment

groups. No clinically important differences in age, sex, body mass index, barostat operating pressure, or pre-drug fasting colonic tone were observed between treatment groups.

CYP2C9 Polymorphism

Genotyping of our study cohort for *CYP2C9* rs1057910 revealed 62 participants with AA and the remaining 10 with CA genotypes. Subjects were equally distributed by genotype across the three treatment groups. As there were no CC homozygotes, who would be expected to have different blood levels of dronabinol in contrast to heterozygotes who had minimal changes in blood levels²⁸, we did not expect clinically significant variations in plasma dronabinol levels or in median area under the concentration curve across treatment groups. Therefore, there would be no impact of individual metabolism of dronabinol on the study endpoints.

Effects of Dronabinol on Colonic Compliance in Overall and Patient Subgroups

There was overall borderline treatment effect on colonic compliance ($p=0.058$), which was most pronounced in the dronabinol 5mg group (Table I). The reduction in Pr_{50} reflects an increase in compliance of the colon in response to dronabinol. In addition, the effect on compliance was prominent in the IBS-D/A subgroup ($p=0.03$ unadjusted for 2 subgroup comparisons, that is, IBS-C and IBS-D/A). Similarly, the effect on compliance within the IBSD/A subgroup was most robust with the 5mg dronabinol dose (Figure 2).

Effects of Dronabinol on Fasting and Postprandial Colonic Tone

Fasting pre-treatment colonic tone was not significantly different among the three groups (Figure 1). There were no significant effects of dronabinol treatment on fasting or postprandial colonic tone (Table I).

Effects of Dronabinol on Phasic Colon Contractile Activity

Phasic contractility during fasting and postprandially was compared for the upper 3 pressure sensors corresponding to the upper descending colon (henceforth called proximal left colon) and separately for the lower 3 pressure sensors corresponding to the junction of descending colon, sigmoid and rectum (henceforth called distal left colon) as recorded in all individuals. Before treatment, fasting colonic motility was not different among the three treatment groups (data not shown).

Dronabinol significantly reduced proximal left colon MI (overall effect $p=0.05$) and tended to reduce post-drug colon MIs (overall effect, $p=0.08$, Table I and Figure 3). In each case, the effect was predominantly attributed to the dronabinol 5mg dose [proximal colon, $p=0.046$ (adjusted) and distal colon, $p=0.13$ (adjusted)].

Overall treatment effects on proximal colon MI were significant in patients with IBS-D/A ($p=0.044$, after adjustment for two tests for the two IBS subgroups), but not in IBS-C (Figure 3), with the predominant effect observed with the dronabinol 5mg dose.

Effects on Colonic Sensory Function during Phasic and Ramp Distensions

Sensation thresholds for gas and pain during ramp distensions were not different among the treatment groups over the entire range of pressures tested (data not shown).

Sensation scores for pain and gas in response to high distension pressures were not significantly different among treatment groups (Table I). No overall treatment effects on mean post-drug VAS sensation rating scores were detected for sensation of gas ($p=0.67$) or pain ($p=0.60$).

Effect on Central Arousal and Stress

Appendix Table 2 shows effects on states of tension, relaxation, energy, and drowsiness, which illustrate the lack of significant central effects of the 2.5 and 5mg dronabinol doses.

Pharmacogenetics: Treatment by Genotype Interaction Effects for the Entire IBS Group

CNR1 rs806378—In the CT/TT genotype (in contrast to the CC genotype), somewhat higher sensation ratings of gas and pain were observed in response to dronabinol versus placebo (Appendix Table 3A, which shows effects of 2.5 and 5mg doses of dronabinol separately). However, significant differential treatment effects were not detected ($p=0.39$ for gas sensation and $p=0.43$ for pain sensation with the pooled analyses of effects of 2.5 and 5mg dronabinol).

In addition, in the CC genotype (but not CT/TT), a more pronounced dronabinol induced reduction in fasting proximal colon MI was observed ($p=0.11$ for CC vs. $p=0.99$ for CT/TT). The effects of *CNR1* rs806378 genotype and dronabinol dose interaction on main sensation and motility endpoints are shown in Table II.

No differential treatment effects on compliance associated with *CNR1* status were detected (interaction, $p=0.93$; treatment effects in CC, $p=0.59$; treatment effects in CT/TT, $p=0.63$).

FAAH rs324420—In the CC genotype, a reduced postprandial tone response was observed during treatment with dronabinol, while in the CA/AA genotype, increased postprandial tone (calculated as the relative change in colonic tone, fasting compared to fed tone) was observed in response to dronabinol (test for drug by genotype interaction, $p=0.10$).

No other differential treatment effects by *FAAH* genetic status were noted (Appendix Table 3A).

MGLL rs4881—No differential treatment effects on motor or sensory functions by *MGLL* rs4881 genotype status were detected.

Treatment by IBS Subgroup by Genotype Interactions (Appendix Table III B and C)

CNR1 rs806378—Treatment effects were suggested with genotype CC in IBS-D/A for compliance ($p=0.066$) and proximal left colon MI ($p=0.075$, Figure 4).

Differential treatment effects among *CNR1* rs806378 genotypes (CC vs. CT/TT) and IBS subtypes were observed for fasting colon tone ($p=0.047$) (see Appendix Table III B and C), with the most pronounced treatment effects observed with the CC genotype in IBS-C ($p=0.09$).

Although the overall test for treatment group by IBS subgroup by *CNR1* genotype interaction was not significant ($p=0.11$), overall treatment effects (i.e. differences among the three treatment groups) on postprandial (relative change from fasting) tone were borderline significant ($p=0.084$, unadjusted) within the IBS-C and CT/TT subgroup, but not for any of the other subtype/genotype combinations.

FAAH rs324420—Differential treatment effects among *FAAH* rs324420 genotypes (CC vs. CA/AA) and IBS subtypes (Figure 5) were observed for proximal left colon MI ($p=0.09$), most pronounced in IBS-D/A and CA/AA ($p=0.013$), and for distal left colon MI ($p=0.046$), most pronounced in IBS-C and CC ($p=0.045$).

***MGLL* rs4881**—The analyses for *MGLL* did not detect any striking “differential” treatment effects, but the minor allele frequency was rather low (1 CC, 12 CT, and 59 TT). There were some suggestions of differential treatment effects for mean VAS gas scores ($p=0.12$), mean VAS pain scores ($p=0.08$), and a possibly higher pain sensation threshold in CT/CC subjects on drug; however, there were only 6 in this subgroup. There also appeared to be an “overall” (irrespective of treatment) modest association of *MGLL* subtype, with relative change in colonic tone and proximal fasting (post-drug) MI.

No other differential treatment effects were associated with IBS and genotype subgroups.

Adverse Effects

The most frequent adverse effects were: drowsy/tired 23%; flushing/hot 19%; headache 13%; dizzy/lightheaded 11%; loopy/foggy thinking 11%; elevated heart rate 11%; relaxed/dream-like state 9%; nausea 8%, dry mouth and eyes 7%. The adverse effects are broken down by group in Appendix Table 4. The only adverse effect which was more common with dronabinol than placebo was loopy/foggy thinking ($p=0.009$ by Fisher's exact test).

DISCUSSION

This study demonstrates cannabinoid modulation of colonic compliance and fasting colonic motility in patients with IBS; specifically, a single dronabinol dose of 5mg acutely increased colonic compliance and reduced fasting colonic motility in the subgroups of IBS-D and IBS-A patients. Previously, dronabinol had been demonstrated to increase colonic compliance and to inhibit colonic motility and tone in healthy male or female volunteers¹⁴. Dronabinol also delayed gastric emptying in female, but not male healthy subjects¹⁵. Our current study involving >90% female IBS patients characterizes dronabinol's effects on colonic motor and sensory functions.

Using a barostat-manometry assembly, we observed an increase in colonic compliance and a decrease in proximal left colon phasic motility index with dronabinol treatment. These two effects are internally consistent and reflect the inhibition of tonic excitatory motor or activation of inhibitory neural mechanisms by the non-selective cannabinoid agonist. In contrast to dronabinol's effects on colonic motor function observed with the 7.5mg dronabinol dose in healthy volunteers¹⁴, the 5mg dose of dronabinol in this study did not significantly inhibit fasting colonic tone or the motor response to feeding in IBS patients. The drug dose is clearly critical, since the 2.5mg dose in this study had no effect, while the 7.5mg dose used previously¹⁴ inhibited colonic tone, increased colonic sensations, and induced central effects such as lightheadedness consistent with known responses to the cannabinoid receptor agonist¹⁴. The observed effects of a single administration of 5mg dronabinol, together with the known expression of cannabinoid receptors on cholinergic neurons in the brain stem, stomach and colon, are consistent with the hypothesis that dronabinol may be inhibiting colonic muscle excitation via cholinergic neurons in the central and enteric nervous systems³⁰. Cannabinoid receptor modulation is a potential target for therapy in diseases associated with accelerated transit²⁹ or increased colonic motor function in patients with IBS-D^{31,32}.

In contrast to the effects of a single dose of 7.5mg dronabinol noted in healthy subjects, a single 5mg dose in IBS patients did not increase stress, arousal, or colonic sensations of gas and pain measured as either sensory thresholds or visual analog ratings in response to random-order phasic distensions. Identification of a peripheral effect on colonic motor function without increasing unwanted sensations of gas or pain, as well as alterations in affect or arousal is critically important to the development of cannabinoid agents as potential therapy in IBS. In agreement with a recent study on dronabinol's effects on visceral

perception to rectal balloon distension³³, our study also did not show any potentially beneficial changes in visceral perception to balloon distension in the left colon.

Our data show that significant effects of dronabinol on colonic compliance and motility were predominantly observed with the 5mg dose in those IBS patients who experience diarrhea (IBS-D and IBS-A). Of note, about 48% of patients with IBS-D have accelerated colonic transit at 24 or 48 hours and, as a group, patients with IBS-A have accelerated transit at 48 hours compared to healthy controls²⁹. Inhibition of colonic motility by dronabinol may provide potential benefit to those IBS-D and IBS-A patients with accelerated transit.

Given the effects of CB₁ receptor modulation on colonic functions¹⁴ and the association of *FAAH* genetic variation with diarrhea and colonic transit in IBS-D patients³⁴, we conducted a pharmacogenetic analysis exploring the influence of genetic variation in the CB₁ receptor and in the rate-limiting catabolic enzymes for anandamide (*FAAH*) and 2-acylglycerol (*MAGL*), the two primary endocannabinoids in humans. Our data suggest that effects of dronabinol on colonic compliance and proximal colonic motility may be influenced by genetic variations in *FAAH* and *CNRI*. We did not observe any significant modulation by variation in *MGLL*, but our analysis of *MGLL* was compromised by the low minor allele frequency of rs4881. Overall, our pharmacogenetic data lend support to the hypothesis that a genetic basis accounts for differences in effects on colonic functions by drugs targeting cannabinoid receptors or the metabolism of anandamide. Therefore, future studies of more selective cannabinoid receptor agonists or antagonists may be more informative if pharmacogenetic analysis is included to help identify individuals more likely to benefit from cannabinoid or anti-cannabinoid medications.

Cannabidiol analogs devoid of the central effects on cannabinoid receptor activation have been proposed as therapy for diarrheal diseases^{35,36}. In contrast, a cannabinoid receptor antagonist may oppose the inhibition of cholinergic mechanisms by endogenous cannabinoids, which may relieve constipation via acceleration of colonic transit and enhancement of intestinal secretion. Izzo et al. showed in a mouse model that the CB₁ antagonist, rimonabant (also known as SR141716A, 0.1–5mg/kg, i.p.), increased defecation, gastrointestinal transit, and fluid accumulation in the colon. These effects were inhibited by atropine (1mg/kg, i.p.), but not by the ganglion blocking agent, hexamethonium, or by antagonists of NK₁ and NK₂ receptors³⁷. Interestingly, in clinical trials of rimonabant used in aiding nicotine cessation or in treating obesity, diarrhea was 2 to 2.4 times more frequent among those treated with the drug than with placebo, suggesting accelerated colonic transit and/or enhanced mucosal secretion resulting from CB₁ blockade^{38,39}.

We did not observe increase in sensation with the 5mg dronabinol dose in IBS, in contrast to the effects of the 7.5mg dose in healthy subjects. This is relevant because any beneficial effects on colonic motor function could potentially be negated by increased sensations of gas or pain. Increased awareness of surroundings was reported more frequently in patients receiving delta-9-THC⁴⁰. On the other hand, Sanson et al.¹⁷ suggested that cannabinoid effects on increasing sensation during colonic distension in rats with inflamed colon were mediated peripherally. Importantly, the increased compliance induced by dronabinol did not compromise our assessment of sensory effects since our study used pressure-based distensions to avoid the erroneous interpretation of sensory changes as would occur with sensory ratings measured using volume-based distensions. Further studies are needed to explore the effects of cannabinoid receptor modulation in the sensory neuraxis in humans after repeated administrations of cannabinoid agents.

The strengths of our study include our research team's extensive experience with methods measuring colonic motility and sensation, and the trial generalizability as shown by sample

size with adequate power to detect clinically meaningful effects on primary endpoints, multiple medication doses studied, analysis by IBS subgroups based on predominant bowel function, and inclusion of pharmacogenetic analysis to assess whether dronabinol's effects may be influenced by genetic variations in cannabinoid signaling or metabolism..

The weaknesses of this study include assessment of a single administration of dronabinol and the non-selective nature of dronabinol for CB₁ and CB₂ receptors. Our study had sufficient power to detect treatment effects on motor and sensory responses based on 24 patients per group; the power was lower for symptom subgroups of IBS and for subgroups based on genotypes, where the number of participants in each group divided according to genotype ranged from 2 to 11. Our study also had limited power to detect differences in sensation thresholds due to the large coefficient of variation in these endpoints when compared to the coefficients for motility indices and sensation ratings. Therefore, the pharmacogenetic results, in particular, are to be viewed as only hypothesis-generating. In addition, knowing the blood levels of dronabinol may also have enhanced the interpretation of the associations of the genetic variations of the effects of dronabinol.

In summary, our study shows that the non-selective cannabinoid receptor agonist, dronabinol, inhibits fasting colonic motility and enhances colonic compliance in IBS, particularly in patients with IBS-D and IBS-A. These effects may be better harnessed with selective cannabinoid receptor agonists and antagonists. A selective CB₁ agonist, in particular, may have potential as therapy in diarrhea-positive IBS patients. Further studies to assess the therapeutic role of dronabinol and other cannabinoid receptor agonists in IBS are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Howlett AC. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* 2002; 54:161–202. [PubMed: 12037135]
2. Matsuda LA, Lolait SJ, Brownstein MJ, et al. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990; 346:561–564. [PubMed: 2165569]
3. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993; 365:61–65. [PubMed: 7689702]
4. Begg M, Pacher P, Batkai S, et al. Evidence for novel cannabinoid receptors. *Pharmacol Ther.* 2005; 106:133–145. [PubMed: 15866316]
5. Database of Single Nucleotide Polymorphisms (dbSNP). National Center for Biotechnology Information, National Library of Medicine; Bethesda (MD: (dbSNP Build ID: {132}). Available from: <http://www.ncbi.nlm.nih.gov/SNP/>
6. Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992; 258:1946–1949. [PubMed: 1470919]
7. Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat.* 2000; 61:3–18. [PubMed: 10785538]

8. Mechoulam R, Benshabat S, Hanus L, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol.* 1995; 50:83–90. [PubMed: 7605349]
9. Sugiura T, Kondo S, Sukagawa A, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun.* 1995; 215:89–97. [PubMed: 7575630]
10. Hinds NM, Ullrich K, Smid SD. Cannabinoid 1 (CB(1)) receptors coupled to cholinergic motoneurons inhibit neurogenic circular muscle contractility in the human colon. *Br J Pharmacol.* 2006; 148:191–199. [PubMed: 16520743]
11. Pinto L, Izzo AA, Cascio MG, et al. Endocannabinoids as physiological regulators of colonic propulsion in mice. *Gastroenterology.* 2002; 123:227–234. [PubMed: 12105851]
12. Izzo AA, Mascolo N, Capasso R, et al. Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. *Naunyn Schmiedebergs Arch Pharmacol.* 1999; 360:221–223. [PubMed: 10494894]
13. Shook JE, Burks TF. Psychoactive cannabinoids reduce gastrointestinal propulsion and motility in rodents. *J Pharmacol Exp Therapeutics.* 1989; 249:444–449.
14. Esfandiyari T, Camilleri M, Busciglio I, et al. Effects of a cannabinoid receptor agonist on colonic motor and sensory functions in humans: a randomized, placebo-controlled study. *Am J Physiol.* 2007; 293:G137–G145.
15. Esfandiyari T, Camilleri M, Ferber I, et al. Effect of a cannabinoid agonist on gastrointestinal transit and postprandial satiation in healthy human subjects: a randomized, placebo-controlled study. *Neurogastroenterol Motil.* 2006; 18:831–838. [PubMed: 16918762]
16. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* 2006; 58:389–462. [PubMed: 16968947]
17. Sanson M, Bueno L, Fioramonti J. Involvement of cannabinoid receptors in inflammatory hypersensitivity to colonic distension in rats. *Neurogastroenterol Motil.* 2006; 18:949–956. [PubMed: 16961698]
18. Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol.* 2005; 5:400–411. [PubMed: 15864274]
19. Talley NJ, Phillips SF, Wiltgen CM, et al. Assessment of functional gastrointestinal disease: the bowel disease questionnaire. *Mayo Clin Proc.* 1990; 65:1456–1479. [PubMed: 2232900]
20. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand.* 1983; 67:361–370. [PubMed: 6880820]
21. MICROMEDEX, H. Micromedex. 2005. Physician's Desk Reference.
22. Hammer HF, Phillips SF, Camilleri M, et al. Rectal tone, distensibility, and perception: reproducibility and response to different distensions. *Am J Physiol.* 1998; 274:G584–G590. [PubMed: 9530161]
23. Viramontes BE, Malcolm A, Camilleri M, et al. Effects of an alpha(2)-adrenergic agonist on gastrointestinal transit, colonic motility, and sensation in humans. *Am J Physiol.* 2001; 281:G1468–G1476.
24. Floyd BN, Camilleri M, Andresen V, et al. Comparison of mathematical methods for calculating colonic compliance in humans: power exponential, computer-based and manual linear interpolation models. *Neurogastroenterol Motil.* 2008; 20:330–335. [PubMed: 17971031]
25. Bharucha AE, Camilleri M, Zinsmeister AR, et al. Adrenergic modulation of human colonic motor and sensory function. *Am J Physiol.* 1997; 273:G997–G1006. [PubMed: 9374695]
26. Delgado-Aros S, Chial HJ, Camilleri M, et al. Effects of a kappa-opioid agonist, asimadoline, on satiation and GI motor and sensory functions in humans. *Am J Physiol.* 2003; 284:G558–G566.
27. Chiang KP, Gerber AL, Sipe JC, et al. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. *Hum Mol Genet.* 2004; 13:2113–2119. [PubMed: 15254019]
28. Sachse-Seeboth C, Pfeil J, Sehrt D, et al. Interindividual variation in the pharmacokinetics of Delta9-tetrahydrocannabinol as related to genetic polymorphisms in CYP2C9. *Clin Pharmacol Ther.* 2009; 85:273–276. [PubMed: 19005461]

29. Camilleri M, McKinzie S, Busciglio I, et al. Prospective study of motor, sensory, psychologic, and autonomic functions in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2008; 6:772–781. [PubMed: 18456567]
30. Sibaev A, Yüce B, Kemmer M, et al. Cannabinoid-1 (CB1) receptors regulate colonic propulsion by acting at motor neurons within the ascending motor pathways in mouse colon. *Am J Physiol*. 2009; 296:G119–G128.
31. Choi MG, Camilleri M, O'Brien MD, et al. A pilot study of motility and tone of the left colon in patients with diarrhea due to functional disorders and dysautonomia. *Am J Gastroenterol*. 1997; 92:297–302. [PubMed: 9040210]
32. Chey WY, Jin HO, Lee MH, et al. Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. *Am J Gastroenterol*. 2001; 96:1499–1506. [PubMed: 11374689]
33. Klooker TK, Leliefeld KE, Van Den Wijngaard RM, et al. The cannabinoid receptor agonist delta-9-tetrahydrocannabinol does not affect visceral sensitivity to rectal distension in healthy volunteers and IBS patients. *Neurogastroenterol Motil*. 2011; 23:30–35. [PubMed: 20718944]
34. Camilleri M, Carlson P, McKinzie S, et al. Genetic variation in endocannabinoid metabolism, gastrointestinal motility and sensation. *Am J Physiol*. 2008; 294:G13–G19.
35. Fride E, Feigin C, Ponde DE, et al. (+)-Cannabidiol analogues which bind cannabinoid receptors but exert peripheral activity only. *Eur J Pharmacol*. 2004; 506:179–188. [PubMed: 15588739]
36. Fride E, Ponde D, Breuer A, et al. Peripheral, but not central effects of cannabidiol derivatives: Mediation by CB(1) and unidentified receptors. *Neuropharmacology*. 2005; 48:1117–1129. [PubMed: 15910887]
37. Izzo AA, Mascolo N, Borrelli F, et al. Defaecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB1 receptors. *Naunyn Schmiedebergs Arch Pharmacol*. 1999; 359:65–70. [PubMed: 9933153]
38. Fernandez JR, Allison DB. Rimonabant. *Curr Opin Investig Drugs*. 2004; 5:430–435.
39. Van Gaal LF, Rissanen AM, Scheen AJ, et al. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet*. 2005; 365:1389–1397. [PubMed: 15836887]
40. Buggy DJ, Toogood L, Maric S, et al. Lack of analgesic efficacy of oral delta-9-tetrahydrocannabinol in postoperative pain. *Pain*. 2003; 106:169–172. [PubMed: 14581124]

Figure 1.
Trial flow chart and baseline characteristics of study participants (Mean $\pm$ SEM, unless otherwise noted).

Figure 2.

Effect of dronabinol on colonic compliance. Data reported are least squares (LS) means and standard errors (SEM). The error bars are based on the square root of the ratio: pooled estimate of residual error variance (across all subgroups from the ANCOVA) divided by the subgroup sample size.

Figure 3.

Effect of dronabinol on colonic phasic pressure activity. Data reported are least squares (LS) means and standard errors (SEM). The error bars are based on the square root of the ratio: pooled estimate of residual error variance (across all subgroups from the ANCOVA) divided by the subgroup sample size.

Figure 4. Pharmacogenetics of *CNR1* rs806378 and colonic motility index. Treatment effects were most prominently suggested in IBS-D/A and the *CNR1* rs806378 genotype CC for proximal left colon MI ($p=0.075$). Data reported are least squares (LS) means and standard errors (SEM). The error bars are based on the square root of the ratio: pooled estimate of residual error variance (across all subgroups from the ANCOVA) divided by the subgroup sample size.

Figure 5. Pharmacogenetics of *FAAH* and colonic motility index. Differential treatment effects among *FAAH* rs324420 genotypes (CC vs. CA/AA) and IBS subtypes were observed for proximal left colon MI, ($p=0.09$), and were most pronounced in IBS-D/A and CA/AA ($p=0.013$). Differential treatment effects were also observed for distal left colon MI ($p=0.046$), and were most pronounced in IBS-C and CC ($p=0.045$). Data reported are least squares (LS) means and standard errors (SEM). The error bars are based on the square root of the ratio: pooled estimate of residual error variance (across all subgroups from the ANCOVA) divided by the subgroup sample size.

Table 1

Effect of Dronabinol on Colonic Compliance, Proximal and Distal Left Colon Motility Index, Fasting and Postprandial (PP) Change in Colonic Tone, and sensation ratings in response to distensions

	Placebo			Dronabinol 2.5mg			Dronabinol 5mg		
	All IBS	IBS-C	IBS-D/A	All IBS	IBS-C	IBS-D/A	All IBS	IBS-C	IBS-D/A
CYP2C9*3:m with CA/AA genotype	3/22	1/8	2/14	3/19	2/10	1/9	3/20	1/10	3/10
Compliance Pr ₅₀ (mL/mmHg)	17.3±0.9	16.6±1.4	17.6±1.0	18.0±1.0	18.5±1.1	17.4±1.4	15.5±1.0	17.0±1.3	14.2±1.2
Fasting tone, mL	105.3±4.8	98.8±7.3	107.1±5.3	107.6±5.2	110.0±6.0	100.3±7.4	109.9±5.3	107.7±7.1	109.1±6.2
PP relative Δ tone (0–30 min) / fasting	30.0±5.0	31.7±7.7	27.1±5.6	32.8±5.5	32.8±6.3	32.5±7.8	32.0±5.5	27.3±7.5	35.9±6.6
Fasting proximal L colon MI	9.0±0.5	8.7±0.8	9.3±0.6	8.9±0.6	8.1±0.6	10.0±0.8	7.6±0.6	7.7±0.8	7.7±0.7
Fasting distal L colon MI	8.0±0.6	7.4±0.9	8.3±0.6	8.3±0.7	7.6±0.8	8.9±0.9	6.8±0.7	5.9±0.9	7.5±0.8
Mean Pain sensation VAS mm	42.5±3.5	45.8±5.4	40.6±3.9	42.8±3.9	44.8±4.5	39.6±5.5	46.1±3.9	44.1±5.2	47.9±4.6
Mean Gas sensation VAS mm	40.0±3.6	42.5±5.6	38.5±4.0	39.9±4.0	41.5±4.6	37.3±5.7	43.1±4.0	41.9±5.4	44.0±4.8

[ml; least square (LS) means ± SEM]

* Note there were no homozygous CC participants for CYP2C9rs1057910 in the entire study; data based on total 75 subjects randomized

Table II

Effects of *CNR1* rs806378 Genotype and Dronabinol Dose Interaction on Main Sensation and Motility Endpoints

Effect	(Mean) Gas Sensation Ratings	(Mean) Pain Sensation Ratings	Proximal Fasting MI	Distal Fasting MI
Gene by treatment (interaction effect)	0.40	0.56	0.48	0.44
CC (n=18 on PLA) overall treatment effect	0.53	0.62	0.037 [†]	0.038 [#]
2.5 mg DRO (n=12) vs. PLA	0.48	0.59	0.85 [†]	0.34 [#]
5 mg DRO (n=14) vs. PLA	0.60	0.59	0.016 [†]	0.064 [#]
CT/TT (n=8 on PLA) overall treatment effect	0.56	0.60	0.67	0.79
2.5 mg DRO (n=11) vs. PLA	0.29	0.36	0.68	0.98
5 mg DRO (n=10) vs. PLA	0.46	0.38	0.69	0.58

Sensation ratings are averaged over the different levels of distension. Total of 73 participants had this genotyping. P-values (unadjusted) from ANCOVA models examining differential treatment effects [placebo (PLA), 2.5mg and 5 mg dronabinol (DRO)]

[†]When the 2.5 and 5mg doses are pooled, these three p-values "collapse" to 0.11.

[#]When the 2.5 and 5mg doses are pooled, these three p-values "collapse" to 0.52.