Neurogastroenterology & Motility

Neurogastroenterol Motil (2012) **24,** 358–e169 doi: 10.1111/j.1365-2982.2011.01874.x

Randomized pharmacodynamic and pharmacogenetic trial of dronabinol effects on colon transit in irritable bowel syndrome-diarrhea

B. S. WONG,* M. CAMILLERI,* D. ECKERT,* P. CARLSON,* M. RYKS,* D. BURTON* & A. R. ZINSMEISTER†

*Clinical Enteric Neuroscience Translational and Epidemiological Research (CENTER), Mayo Clinic, Rochester, Minnesota, USA
†Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA

Abstract

Background *Genetic variation in endocannabinoid metabolism is associated with colonic transit in irritable bowel syndrome (IBS) with diarrhea (IBS-D). The nonselective cannabinoid (CB) receptor agonist, dronabinol (DRO), reduced fasting colonic motility in nonconstipated IBS. FAAH and CNR1 variants influenced DRO's effects on colonic motility. Our aims were: (i) to compare dose-related effects of DRO to placebo (PLA) on gut transit in IBS-D, and (ii) to examine influence of genetic variations in CB mechanisms on DRO's transit effects.* ***Methods*** *Thirty-six IBS-D volunteers were randomized (double-blind, concealed allocation) to twice per day PLA (n = 13), DRO 2.5 mg (n = 10), or DRO 5 mg (n = 13) for 2 days. We assessed gastric, small bowel, and colonic transit by validated radioscintigraphy and genotyped the single nucleotide polymorphisms CNR1 rs806378 and FAAH rs324420. Data analysis utilized a dominant genetic model.* ***Key Results*** *Overall treatment effects of DRO on gastric, small bowel, or colonic transit were not detected. CNR1 rs806378 CT/TT was associated with a modest delay in colonic transit at 24 h compared with CC (P = 0.13 for differential treatment effects on postminus pretreatment changes in colonic transit by genotype). No significant interaction of treatment with FAAH rs324420 was detected.* ***Conclusions & Inferences*** *Overall, DRO 2.5 or 5 mg twice per day for 2 days had no effect on gut transit in IBS-D. There appears to be a treatment-by-genotype effect, whereby DRO preferentially delays colonic transit in those with the CNR1 rs806378 CT/TT genotypes. Further study of CB pharmacogenetics may help identify a subset of IBS-D patients most likely to benefit from CB agonist therapy.*

Keywords *anandamide, cannabinoid, fatty acid amide hydrolase, gastric, motility, nonselective, receptor, small bowel.*

INTRODUCTION

Cannabinoid receptors type 1 (CB_1) are identified in colonic mucosa and neuromuscular layers[1–3]; they are also expressed in plasma cells and influence mucosal inflammation.[4] The endocannabinoid system consists of CB_1 and CB_2 receptors; the ligands of these receptors are anandamide and 2-arachidonyl glycerol (2-AG), and their respective ligand-inactivating enzymes are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGLL).[5–8] Activation of CB_1 receptors coupled to cholinergic motor neurons inhibits excitatory nerve transmission in human colonic circular muscle *in vitro*.[9] *In vivo*, endocannabinoids acting on myenteric CB_1 receptors tonically inhibit colonic propulsion in mice[10]; they also inhibit gastric and small intestinal transit without altering intraluminal pressure or basal tone in rodents.[11,12]

Previously, we have shown that dronabinol (DRO), a nonselective CB receptor agonist, inhibits gastric emptying and colonic motility in healthy humans.[13,14] The effects on colonic tone and phasic motility were observed with 7.5 mg DRO, which also induced side effects of drowsiness, lightheadedness, and dizziness.[13] The 5 mg DRO dose was more tolerable among healthy participants.[14] In the patients with irritable

Address for Correspondence
Michael Camilleri, Mayo Clinic, Charlton 8-110, 200 First Street S.W., Rochester, MN 55905, USA.
Tel: +1507 266 2305
e-mail: camilleri.michael@mayo.edu
Received: 18 October 2011
Accepted for publication: 19 December 2011

bowel syndrome (IBS) with diarrhea (IBS-D), genetic variation in endocannabinoid metabolism in the fatty acid amide hydrolase (*FAAH*) gene was associated with symptoms and colonic transit.[15] In another prior study of nonconstipated IBS patients consisting of both patients with IBS-D and IBS with alternating bowel habit (IBS-A), DRO 5 mg reduced fasting colonic motility.[16]

We hypothesized that DRO inhibits colonic transit in IBS, and that these inhibitory effects are modulated by variations in the genes for the CB_1 receptor (*CNR1*) and for *FAAH*, the rate-limiting enzyme in degradation of the endocannabinoid anandamide. Our specific aims were: (i) to compare the effects of two consecutive days of twice per day administration of oral placebo, DRO 2.5 mg, and DRO 5 mg on gastric, small bowel, and colonic transit in cannabinoid (CB)-naive IBS-D patients; and (ii) to examine the potential influences of genetic variations in *CNR1* and *FAAH* on the transit effects of DRO treatment.

MATERIALS AND METHODS

Study design

This was a double-blind, randomized, placebo-controlled, parallel-group study (registered at ClinicalTrials.gov, identifier NCT01253408) of the pharmacodynamic effects of DRO on gastric, small bowel, and colonic transit of otherwise healthy participants with IBS-D by Rome III criteria. Their ages were between 18 and 69 years, and body mass indices between 21 and 56 kg m^{-2}, with two subjects with BMI > 40 kg m^{-2}. The study was conducted in the Clinical Research Unit at Mayo Clinic in Rochester, MN (NIH CTSA grant RR0024150), where the full trial protocol is kept; the study began on October 2008 and was completed on April 2011. 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 patients with IBS who reside within 150 miles of Rochester, MN. Participants completed a validated bowel disease questionnaire (BDQ, including questions that correspond to Rome III criteria)[17] and the Hospital Anxiety and Depression Inventory (HAD).[18] Potential participants who met the eligibility criteria for the study underwent a complete history and physical examination before enrollment. All candidates were screened by history and review of their medical records to ensure they were CB-naïve. All females of childbearing potential had to have a negative pregnancy test within 48 h of study. The trial flow is summarized in Fig. 1.

Experimental design

Thirty-six participants with IBS-D were enrolled and completed most studies as in the protocol with measurements over 48 h. Participants were randomized to oral administration of matching placebo, DRO 2.5 mg, or DRO 5 mg, taken with water twice per day for 2 days, with the morning dose ingested at the study center under supervision of study staff.

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

Pharmacology of DRO

Dronabinol is a synthetic delta-9-tetrahydrocannabinol (Δ^9-THC). It is a CB agonist that is nonselective, with affinity for both CB_1 and CB_2 receptors. It is highly (~95%) absorbed after a single oral dose[19]; however, due to high first pass hepatic metabolism (primarily by microsomal hydroxylation) and lipid solubility, only 10–20% of administered oral doses reach the systemic circulation. The onset of action is at 0.5–1 h after oral administration, and the peak effect is at 2–4 h. The elimination phase follows a two-compartment model, with an initial half-life of approximately 4 h and a terminal half-life of 25–36 h. Biliary excretion is the major route of elimination. The rapid onset and peak effect of the

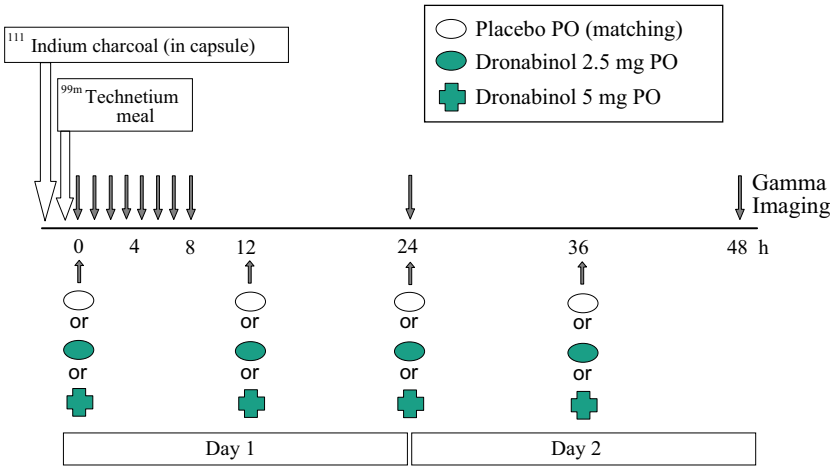


Figure 1 Experimental design showing timing of study medications and scintigraphic transit measurements.

medication and the experimental nature of the study with potential for adverse effects (mental, dizziness, etc.) led us to choose an experimental design that required only 2 days of treatment to observe the acute effects of DRO, rather than 7–10 days of treatment, which would be required for drug levels to reach steady state. Given the well-published pharmacokinetic results[19] and the prior observations of pharmacological effects of DRO using this acute administration design,[13,14,16] we did not conduct any blood measurements for drug levels.

Experimental procedure

All participants underwent the following procedures: documentation of eligibility, screening questionnaires, and physical examination within the prior month. The physical examination included standard rectal and pelvic floor examinations[20] to exclude rectal evacuation disorder. This was deemed necessary to ensure the diarrhea was not secondary to ''retention of stool with overflow''. Participants then underwent baseline colonic transit measurement (GC 24 and GC 48 h), off treatment. Treatment days corresponded to the scintigraphic transit testing days (days 1 and 2) with participants receiving the medication to which they were randomized. Scintigraphic measurements of gastric, small bowel, and colonic transit were conducted, using a previously validated method (see elsewhere in the text) on days 1 and 2, and were completed with a fasting 48-h scan on day 3 when no medication was administered.

On days 1 and 2, the morning dose of medication was ingested in the research laboratory, with the participant fasting. On day 1, the morning dose of medication was administered together with the delayed release capsule containing ^{111}In-labeled activated charcoal used to measure colonic transit. On day 2, the morning dose of medication will be given after the 24-h scan. The evening doses on days 1 and 2 were ingested by participants at bed time in their homes. This timing was selected to reduce the potential for adverse effects such as drowsiness and dizziness, which we previously observed in humans who were administered similar doses.

With appropriate consent, a venous blood sample was obtained from all participants, except one from whom a blood sample could not be drawn despite multiple unsuccessful venipuncture attempts, for DNA extraction and pharmacogenomics studies.

Selection of candidate endocannabinoid genetic polymorphisms

To address the impact of pharmacogenetics on the transit response to DRO, we selected two genetic variants, based on the findings in our prior studies[15,16] and the minor allele frequencies observed in our database. FAAH and CB_1 receptor expressions have been localized to myenteric neurons.[21]

1. *CNR1* is the gene coding for the CB_1 receptor. *CNR1* rs806378 (CC vs CT/TT) showed potential effect on fasting proximal left colonic motility in IBS-D and IBS-A patients.[16] The T allele of *CNR1* polymorphism rs806378 is associated with altered nuclear protein binding in an electrophoretic mobility shift assay, suggesting that rs806378 is a functional polymorphism.[22] We chose to study rs806378 (whose nearest gene on the chromosome is *CNR1*), as we previously demonstrated association of this variant with gastric motor functions. In a study by Vazquez-Roque *et al.*,[23] rs806378 CC genotype was associated with reduced fasting gastric volume, as well as a modest, nonsignificant association with gastric emptying of solids compared to the CT/TT group.

2. *FAAH* is the rate-limiting enzyme for metabolism of the endocannabinoid, anandamide. The 385C-to-A allelic variant of rs324420 in the human *FAAH* gene leads to a Pro129Thr amino acid change, which decreases expression of the FAAH protein secondary to reduced protein stability.[24] The prevalence of A allele is 16–25% in studies of Caucasians in the NCBI database. Previously, our laboratory confirmed the A allele frequency to be 25% in our sample population of healthy controls and IBS patients in southeastern Minnesota.[15] Reduction in FAAH protein level and activity compromises inactivation of the endocannabinoid anandamide. This leads to higher synaptic concentrations of anandamide and, hypothetically, a greater effect of the exogenously administered CB, DRO, via activation of CB_1 and CB_2 receptors.

Genotyping

DNA was extracted from whole blood, as previously described.[24] Genotyping of *FAAH* rs324420 and *CNR1* rs806378 was performed using Taqman™ SNP Genotyping assays (Applied Biosystems, Inc., Foster City, CA, USA) in accordance with manufacturer instructions.

Gastrointestinal and colonic transit

A validated scintigraphic method was used to measure gastric, small bowel, and colonic transit. A methacrylate-coated capsule that dissolves in the alkaline pH of the distal ileum was used to release ^{111}In-labeled activated charcoal particles to evaluate colonic transit on sequential scans.[25] Orally ingested ^{99m}Tc-labeled egg meal allows measurement of gastric and small bowel transit. The method has been shown to detect accelerated or delayed transit. These measurements of colonic transit are associated with altered stool frequency and consistency and are predictive of response to therapy for bowel dysfunction.[26–29] Three standard meals were ingested during day 1, and patients were instructed to eat their normal diet on day 2.

The primary endpoint for analysis was colonic transit geometric center at 24 h. Secondary endpoints were colonic transit geometric center at 48 h, ascending colon emptying $T_{1/2}$; gastric emptying $T_{1/2}$; and colonic filling at 6 h (a surrogate for small bowel transit time).

Sample size assessment

Table S1 (in Supporting information) shows the coefficients of variation (COV) and effect sizes demonstrable with approximately 80% power, assuming an average $n = 12$ per group, using a two-sample t-test with a two-sided α level of 0.05.

Statistical analysis

The entire research team was blinded to treatment allocation until all studies 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 mean for the remaining subjects. An analysis of covariance (ANCOVA) was used to assess treatment effects on colonic transit incorporating BMI and the corresponding ''baseline'' 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 pharmacogenetic ANCOVA models that explored

gene-by-treatment interactions, one patient randomized to DRO 2.5 mg was excluded as she did not consent to provide a DNA sample.

RESULTS

Participants and compliance with medication

The trial flow is shown in Fig. 1. Forty-six patients were assessed for eligibility. Thirty-six IBS volunteers meeting the entry criteria were screened, randomized, and completed the study. A total of 13 volunteers randomly received placebo BID, 10 received DRO 2.5 mg BID, and 13 received DRO 5 mg BID. One patient in the DRO 5 mg group discontinued medication due to an adverse event, and those data were imputed in accordance with the statistical analysis plan (see elsewhere in the text). The table within Fig. 2 summarizes patient demographics by treatment groups. No clinically important differences in age, sex, and body mass index were observed between treatment groups.

Pharmacodynamic effects of DRO on gastric, small bowel and colonic transit

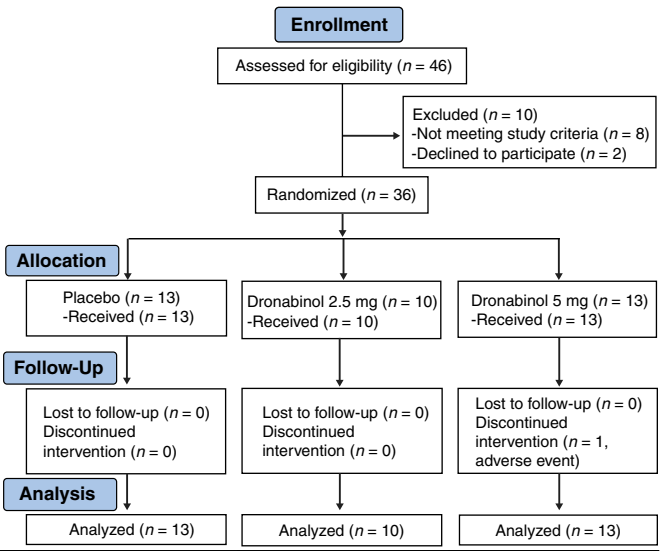
There were no overall or dose-related treatment effects on gastric ($P = 0.88$), small bowel ($P = 0.76$), and colonic (overall $P = 0.23$; 2.5 mg DRO vs placebo, $P = 0.16$; 5 mg DRO vs placebo $P = 0.53$) transit (Fig. 3A,B).

Pharmacogenetics: treatment by genotype interaction effects for the entire ibs group

In the CC *FAAH* rs324420 genotype group the baseline values were fairly similar. There were no qualitative differences for changes (Post-Pre) in colonic transit from pretreatment baseline values following placebo or DRO treatment (Fig. 4A). However, the baseline GC 24 h values in the CA/AA subgroup were rather dissimilar. In this subgroup, although there appear to be differential treatment effects (e.g., placebo vs 2.5 or 5 mg doses), a statistically significant treatment-by-gene interaction was not detected ($P = 0.46$).

CNR1 rs806378 CC genotype was associated with numerical post-treatment changes in colonic transit GC24 from pretreatment that were qualitatively similar in the three treatment groups (Fig. 4B). In contrast, the CT/TT genotype subgroup showed slowing of colonic transit post-treatment with both 2.5- and 5-mg doses, in contrast to the placebo group, which showed a slight acceleration post-treatment. The statistical analysis revealed no significant differences to suggest a gene-by-treatment dose interaction.

Given the similarity in the slowing effects of the DRO 2.5- and 5-mg doses in the *CNR1* rs806378 CT/TT genotype group, we examined potential differential treatment effects associated with genotype comparing placebo with the combined DRO dose groups. As illustrated in Fig. 5, the numerical slowing of colonic transit at 24 h (indicated by individual patient data in



Age (years)	36.7 ± 3.1	47.7 ± 7.9	42.3 ± 4.5
# Females:Males	13:0	10:0	11:2
BMI (kg m^{-2})	30.8 ± 1.8	28.7 ± 1.5	31.6 ± 3.9

Figure 2 Trial flow chart and baseline characteristics of study participants (mean ± SEM, unless otherwise noted).

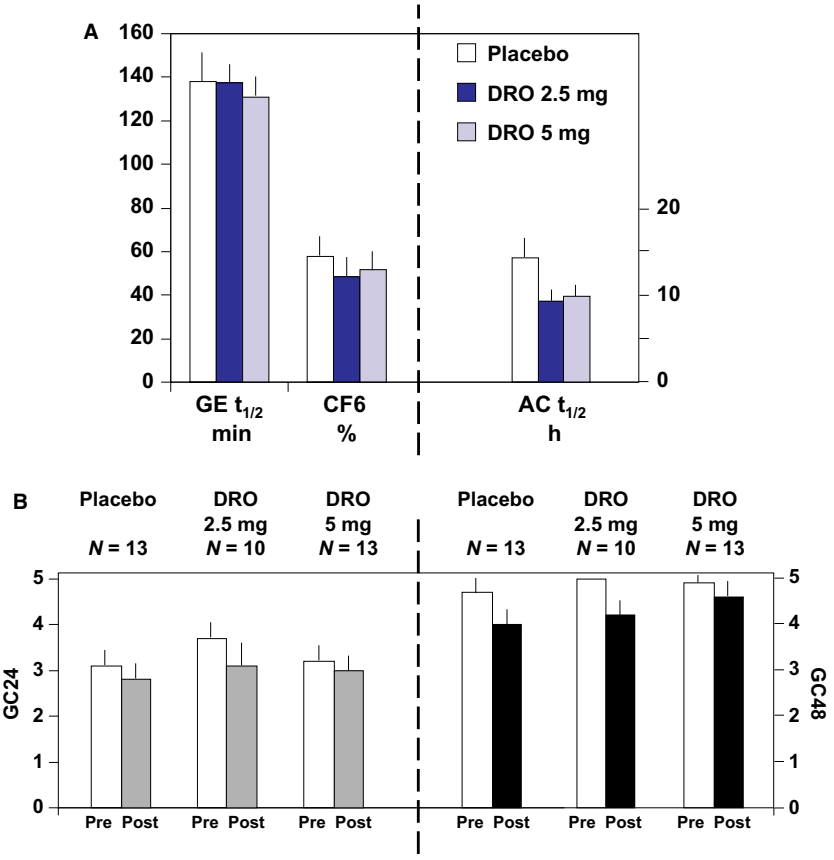


Figure 3 (A) Effect of dronabinol (DRO) on gastric emptying, colonic filling, and ascending colon emptying $t_{1/2}$. Data show mean ± SEM. (B) Effect of DRO on colonic transit expressed as geometric center at 24 and 48 h (GC24 and GC48, respectively). Data show mean ± SEM. The predrug GC48 for DRO 2.5 mg has no SEM bar because all measurements reached a maximum of 5 GC units.

Fig. 5A, and by the post-pre change in colonic transit at 24 h in Fig. 5B) was confirmed in the CT/TT genotype group, relative to placebo. While this was not statistically significant (P = 0.14 for delta GC 24), the unexpected lack of correlation in pre vs post-treatment GC 24 values resulted in larger than anticipated variation for changes in colonic transit.

Analysis of *FAAH* rs324420 genotype for differential treatment effects on post-treatment minus pretreatment colonic transit at 24 h was not significant (test for gene-by-treatment interaction, P = 0.47).

Adverse effects

The observed adverse effects (Table S2) were not significantly different among the three treatment groups.

DISCUSSION

Cannabinoid receptors are located on cholinergic neurons in the brain stem, stomach, and colon. Their activation by the nonselective CB receptor agonist, DRO, inhibits gastrointestinal and colonic muscle excitation by inhibiting cholinergic neurons in the central and enteric nervous systems.[30] Our overall hypothesis was that CB receptor modulation is a potential target for therapy in diseases associated with accelerated transit,[27] as in the increased colonic motor function observed in patients with IBS-D.[31,32] About 48% of patients with IBS-D have accelerated colonic transit at 24 or 48 h compared with healthy controls.[27] Given the effects of CB_1 receptor modulation on colonic functions[13,16] and the association of *FAAH* genetic variation with diarrheal symptoms and colonic transit in IBS-D patients,[24] we conducted a pharmacogenetic analysis exploring the influence of genetic variations in the CB_1 receptor and in the rate-limiting catabolic enzyme for anandamide (FAAH) in humans.

This study demonstrates that nonselective CB receptor stimulation with DRO 2.5 or 5 mg b.i.d. does not significantly alter overall gastric, small bowel, or colonic transit in patients with IBS-D, of whom the vast majority (34/36) in the current study was female. In a previous study of gastrointestinal and colonic transit in 30 healthy volunteers (seven males and eight females randomized in each of two groups to DRO and placebo), we had evaluated the effects of DRO 7.5 mg b.i.d and observed significant delay of gastric emptying in females,[14] but no significant effects on small bowel or

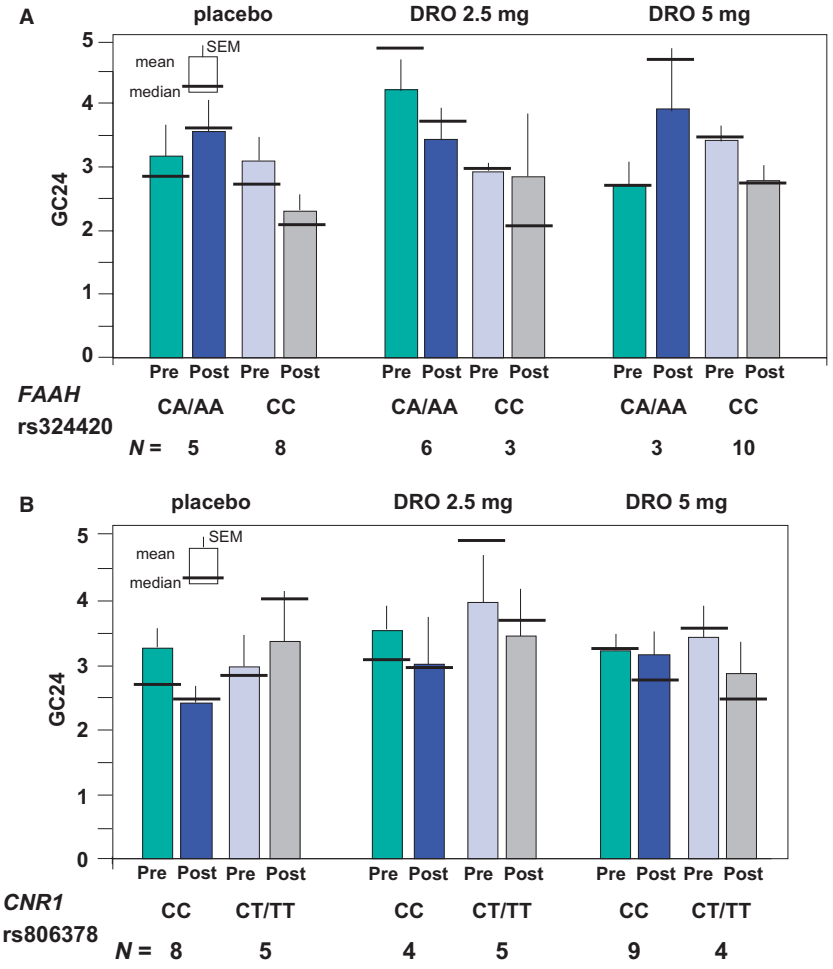


Figure 4 (A) Pharmacogenetics of *FAAH* rs324420 and effects of the two doses of dronabinol (DRO) and placebo on colonic transit at 24 h (GC24). Data show mean, SEM, and median. (B) Pharmacogenetics of *CNR1* rs806378 and effects of the two doses of DRO and placebo on colonic transit at 24 h (GC24). Data show mean, SEM, and median.

colonic transit in either gender.[13] Therefore, the effect of DRO in IBS-D patients on the primary endpoint of interest (overall colonic transit) is consistent with the effect observed in healthy human subjects. The absence of an effect on gastric emptying in IBS-D patients in the current study may reflect the lower dose of DRO used (5 mg b.i.d. instead of 7.5 mg b.i.d.).

The current study results do not conform with the observed reduction in intraluminally measured colonic motility and compliance observed with DRO treatment of patients with nonconstipated IBS[16]; however, it is worth noting that the 5 mg DRO inhibited fasting colonic phasic pressure activity and colonic compliance, and there was no demonstrated effect on postprandial colonic tone or phasic pressure activity.[16] Overall, the two studies question whether a nonselective CB agonist that has potential for central adverse effects can be dosed at sufficient high levels to replicate colonic motor inhibitory effects observed with 7.5 mg DRO b.i.d. Clearly, peripherally selective, CB1 modulating agents are required to explore the role of CB mechanisms in the control or modulation of colonic motor function.

On the other hand, the pharmacogenetic component of the study shows that, even with the small numbers of patients involved in the genotype subgroups, some patients who carry the *CNR1* rs806378 CT/TT genotype may show notable delays in colonic transit with DRO treatment.

In the current study, we elected to use the lower maximum dose of 5 mg DRO b.i.d. because of the central side effects of drowsiness and dizziness observed in our prior study with a single dose of 7.5 mg DRO in healthy subjects.[13] In contrast, a single 5 mg dose in IBS patients did not increase stress or arousal in another previous study in our laboratory.[14] We perceived that it was important to limit the maximum dose to 5 mg DRO b.i.d., as this was associated with tolerable central effects in prior studies,[14] and it had previously been shown that a single 5-mg dose of DRO acutely increased colonic compliance and reduced fasting colonic motility in the

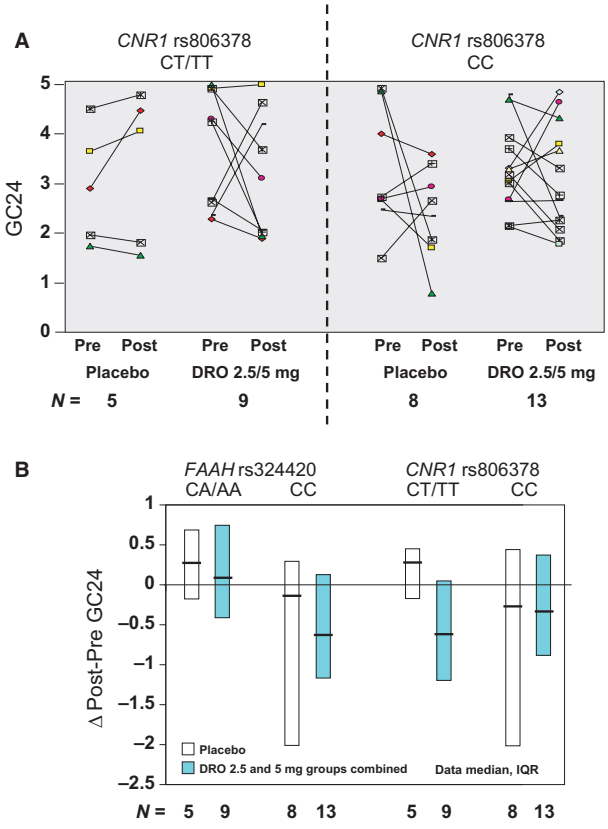


Figure 5 Pharmacogenetics of *FAAH* rs324420 and *CNR1* rs806378 on pre and post-treatment colonic transit GC24 for each individual participant (A) and the change in colonic transit at 24 h (B) for the combined dronabinol treatment group compared with placebo.

subgroups of IBS-D and IBS-A patients.[16] The same 5 mg DRO dose also increased colonic compliance and decreased colonic motility and tone in healthy male and female volunteers.[13] In fact, the differential effect of DRO (combined 2.5 and 5 mg groups) relative to placebo treatment on median colonic transit is about 0.80 geometric center units at 24 h in the *CNR1* rs806378 CT/TT genotype group. This difference in GC24 with DRO relative to placebo is consistent with the magnitude of effects of other agents that significantly affect bowel function, such as linaclotide in patients with IBS-C[33] or alosetron in IBS-D patients, in terms of the difference between post- and pretreatment colonic transit.[34]

The lack of demonstrable effects of genetic variation in *FAAH* (rs324420) on transit in response to DRO is not known. However, in contrast to the significant modulation of the effect of DRO by changes in the function of the CB_1 receptor due to variation in *CNR1*, it is conceivable that the *FAAH* alteration results in only a small additional variation in the level of endocannabinoids at the synapse. In accordance with this hypothesis, the variation in endocannabinoids is not sufficient to modify the overall or combined effects of CB agonist (DRO) and the endocannabinoids reaching the CB_1 receptors.

Our study illustrates the potential for CB agents to modulate colonic function in IBS-D, although this may only pertain to a subgroup of patients, based on a variation in the gene for the CB_1 receptor. It is possible that greater peripheral selectivity of the CB_1 agonist may have greater effects on transit, and that selection of IBS patients based on *CNR1* rs806378 CT/TT genotype may enhance the effects of agents acting on CB_1 receptors. The potential for CBs to change gastrointestinal and colonic transit is illustrated by the effects of experimental, more peripherally selective agents. Thus, *in vivo* studies in mice by Storr *et al.*[35] demonstrated that the inverse CB agonist, AM251, accelerated upper gastrointestinal transit and whole gut transit, whereas the neutral CB antagonist, AM4113, also increased upper gastrointestinal transit. Regional effects of CB antagonists also differ in mice, with reduced colonic expulsion with both AM251 and AM4113; whereas, whole gut transit is accelerated by both agents at specific dose levels.[35] Although there are data suggesting that CB modulation with the CB_1 and CB_2 receptor antagonists[36] and the FAAH inhibitor, AM3506,[37] may affect visceral sensation in inflammation models[36] or gastrointestinal transit and colonic fecal output in mice exposed to endotoxin,[37] the effects on pain or visceral sensation in human studies have been disappointing.[38,39]

The strengths of our study include the validated methods measuring gastrointestinal and colonic transit, the clinical significance (number and consistency of bowel movements) of the measurement used as primary endpoint (that is colonic GC24), and inclusion of pharmacogenetic analysis to assess whether DRO's effects may be influenced by genetic variations in CB signaling or metabolism.

The weaknesses of this study include assessment of only four doses of DRO over 2 days and the nonselective nature of DRO for CB_1 and CB_2 receptors. Importantly, our study had insufficient power to detect gene-by-treatment interactions. We conducted a *post hoc* analysis of the statistical power of the study based on the variation in the primary response measure (GC at 24 h), the sample sizes of each group based on specific genotypes, and the pattern of delta GC24 h data among the four gene-by-drug combinations. Using the dominant genetic model (homozygous major vs combined heterozygous plus homozygous minor groups), treatment group categorized as placebo compared to the combined 2.5- and 5-mg groups, and the pattern of

median values in Fig. 5B, there would have been approximately 80% power to detect gene-by-treatment interactions of a magnitude illustrated in Table S3, if the variation in delta (post-pre) GC24 values would have been the same as the variation in baseline GC24 values (SD = 1.04). Unfortunately, the observed variation in delta GC24 values was greater (SD = 1.44) and, as a result, there was insufficient power to detect a potential gene-by-treatment interaction. In fact, the usual advantage gained from using baseline GC24 as a covariate did not manifest itself in this study. In summary, the analysis for differential drug effects depending on genotype was limited both by the sample sizes, as well as by the larger than expected observed variation in delta GC24. Thus, there was only 52% power to detect the ''interactions'' illustrated by the patterns in Table S3, given the observed variation in delta GC24. To achieve 80% power with the observed SD in GC24 of 1.44, we would require approximately 40 patients per treatment arm assuming a similar distribution of genotypes in each arm (Table S3). This information is helpful to plan future studies.

Therefore, the pharmacogenetic results in particular are to be viewed as hypothesis generating. However, the individual responses in the patients who carry the *CNR1* rs806378 CT/TT genotype suggest that inclusion of pharmacogenetics in drug appraisal is relevant, at least at the proof-of-concept stage with quantifiable endpoints.

In summary, our study shows that the nonselective CB receptor agonist, DRO, does not significantly affect colonic transit; however, DRO may inhibit colonic transit in a subset of IBS-D patients, based on a specific genetic variation in CB_1. A selective CB_1 agonist may have potential as therapy in diarrhea-predominant IBS patients. Further studies to assess the therapeutic role of selective CB receptor agonists in IBS are warranted. Clinical trials may be enhanced by inclusion of stratification based on *CNR1* rs806378 genotype or use of the genotype variation as a covariate in the analysis of results.

ACKNOWLEDGMENTS

The authors wish to thank Mary Lempke, PharmD, research pharmacist, and Mrs. Cindy Stanislav, secretary, for assistance.

FUNDING

This work was funded by grant RO1-DK079866 from National Institutes of Health (to Dr. Camilleri) and Mayo Clinic CTSA grant (RR24150).

DISCLOSURES

No competing interests declared.

AUTHOR CONTRIBUTION

B. Wong participated in patient recruitment and screening, data collection, writing protocol and manuscript; M. Camilleri performed study conceptualization, writing of protocol, and manuscript; D. Eckert recruited patients; P. Carlson performed genotyping; M Ryks was involved in data collection; D. Burton performed data processing; A.R. Zinsmeister participated in database management and statistical analysis.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Sample size assessment based on observed variation in this study.

Table S2. Adverse events recorded.

Table S3. Poststudy assessment of statistical power.

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