Study Title: GI Permeability Change in Response to Aquamin®
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SUB-STUDY TITLE: DIRECT MEASUREMENT OF GASTROINTESTINAL PERMEABILITY CHANGE IN RESPONSE TO AQUAMIN®

INTRODUCTION

This protocol is for a study in which we will measure gastrointestinal permeability in healthy adult subjects, subjects with ulcerative colitis (UC), and subjects with diarrhea predominant irritable bowel syndrome (IBS-D) before and after Aquamin® ingestion. This protocol should be considered as a sub-study to our ongoing IRBMED-approved clinical trial entitled: *Aquamin®, a multi-mineral natural product from red marine algae, as an adjuvant intervention for mild ulcerative colitis and ulcerative colitis in remission (HUM00156676)*. Like the parent study, this sub-study protocol will also be submitted to the FDA under IND 141600. In the existing trial, individuals with ulcerative colitis (UC) are receiving Aquamin® in the hope that the multi-mineral supplement will improve barrier function in the gastro-intestinal tract and, thereby, help reduce inflammation and improve symptomatology.

The gastrointestinal barrier consists of various protective components, including pancreatic and gastric juices, commensal microbiota preventing pathogenic colonization, the mucosal layer, and the epithelial cell layer held together by tight junction proteins. Intestinal permeability may be measured through ingestion of a molecule which is detectable and quantifiable in excreted urine. Gastrointestinal tract dysfunction has been implicated in a number of disease states, including inflammatory bowel disease and functional bowel disorders, or disorders that alter the brain-gut axis, such as irritable bowel syndrome. While UC is, undoubtedly, an inflammatory disease, there is a large body of information suggesting that defects in the gastrointestinal tract barrier may be part of the underlying pathophysiology (1-5). Similarly, studies have demonstrated increased permeability in patients with IBS-D (6-9).

Of the studies indicating pathophysiological involvement of alterations in permeability in IBS patients, one of them collected stool samples from IBS-D patients, which showed high serine protease activity (10,11). When fecal extract with this elevated serine protease activity was infused into the colon of mice, the researchers observed increased colonic permeability (10,11). The researchers then added serine protease inhibitors, which reversed the permeability effect (10,11). Other changes in the gut barrier have been observed with changes in the intercellular junctions of the intestines (12); mast cell changes, likely from tryptase release, are also seen to increase permeability in IBS-D patients (13-15). These changes, among others not listed here, support the theory of increased gut permeability in IBS-D patients, which would make IBS-D patients a particularly interesting cohort of comparison against ulcerative colitis patients in this study (6). Aquamin® may also help reduce inflammation and improve symptomatology in both ulcerative colitis patients in remission and in IBS-D patients.

Studies from our laboratory conducted with human colon tissue in organoid culture have demonstrated that Aquamin® up-regulates multiple epithelial cell surface proteins that contribute to barrier formation. We have seen this in tissue from healthy subjects as well as with tissue from individuals with UC (16,17). Increased barrier protein expression was accompanied by increased trans-epithelial electrical resistance (a measure of permeability control) and with increased tissue cohesion (18). In parallel with these findings, the results from our recently-completed Aquamin® interventional trial with healthy individuals demonstrated up-regulation of several of the same barrier proteins as seen *in vitro* (19-21). Taken together, these

data strongly suggest that Aquamin® has the potential to improve barrier function in the gastrointestinal tract and that this may be part of its mechanism of action in individuals with UC; given the barrier permeability differences in IBS-D patients, Aquamin® also will likely improve barrier function and subsequently help ameliorate symptom burden in patients with IBS-D. In spite of this, there has not yet been an attempt to directly assess gastrointestinal barrier function *in vivo* in response to Aquamin® intervention (neither in healthy individuals nor in subjects with any inflammatory bowel disease). This is also not a part of the ongoing trial with UC sufferers. The goal of the proposed study is to address this issue. Our proposal is to enroll 12 subjects who are healthy adults, 12 subjects with UC in remission, and 12 subjects with IBS-D and provide 90-days of intervention with Aquamin® under conditions identical to those currently being used as part of the UC trial. Before and after intervention, we will use the lactulose:mannitol ratio in urine (22) as a way to assess effects of intervention on a direct measure of gut permeability.

Study aim: To determine whether Aquamin® taken daily, standardized to 800 mg of calcium per day for 90 days, will improve barrier function in the gastrointestinal tract as assessed using the lactulose:mannitol ratio in urine in healthy subjects, ulcerative colitis subjects, and diarrhea predominant irritable bowel subjects.

The primary outcome is an assessment of the barrier function, evaluated by a change in the lactulose:mannitol ratio in urine by comparing values at 90-days (post-intervention) to baseline (pre-intervention) levels. Pre- post-values will be measured and compared using appropriate statistical methods performed by the study biostatistician and the program GraphPad Prism, version 8.0 will also be used.

Approach:

Healthy Subjects: We will recruit and enroll 12 healthy subjects (i.e., not having UC) for this study. The inclusion and exclusion criteria for the healthy subjects is listed below.

Subjects with UC: We will recruit and enroll 12 subjects with UC. The inclusion and exclusion criteria listed in Section 4.5 and 4.6 (of the parent study and is listed below) will be used except use of anticoagulants will not be an exclusion.

Subjects with IBS-D: We will recruit and enroll 12 subjects with diarrhea predominant irritable bowel syndrome. The inclusion and exclusion criteria for the IBS-D subjects is listed below.

Aquamin®: The study agent will consist of Aquamin® capsules. The capsules will be identical to those being used in the parent study. Subjects will receive an amount of Aquamin® sufficient to provide 800 mg of calcium per day. See Section 5 of the parent study for details of the study agent. Please note that in the proposed study, there will be no placebo group.

Inclusion Criteria for the healthy subjects:

- Must be able to give written informed consent.

- Be generally healthy, male or female, ages 18 to 80 years old.

- A negative pregnancy test for pre-menopausal women with intact female reproductive organs. The negative pregnancy test must be within 2 weeks of the baseline visit and the subject must agree to use appropriate birth control over the study period. Acceptable forms of

birth control include: hormonal (such as pill, IUD), barrier (such as condom, diaphragm), surgical, or abstinence. Post-menopausal is defined as no menses for the previous 12 months. If cessation of menses is within 12 months, then the subject should be treated as pre-menopausal and a pregnancy test performed.

Exclusion Criteria for the healthy subjects: The exclusion criteria are similar to the one listed in Section 4.6 for UC subjects (of the parent study) with few exceptions.

- Must not be pregnant or lactating; patients of childbearing potential unwilling to use acceptable birth control throughout the study also excluded.

- Must not be participating in any other interventional trial using an investigational drug.

- Subjects likely to be uncooperative or unable to comply with study procedures

- Participants must not have a history or diagnosis of any of the following conditions:

    - i. Crohn's disease, other inflammatory bowel disease, or a functional gastrointestinal disorder, including irritable bowel syndrome.

    - ii. Any stomach or intestinal bleeding disorders (gastrointestinal bleeding from gastric or duodenal ulcers, or gastrin secreting tumors) or active gastric / duodenal ulcers - peptic ulcer disease (without bleeding in last 3 months).

    - iii. Any gastrointestinal or colonic malignancy.

    - iv. Kidney disease, including kidney "stones" or hypercalcemia.

    - v. Coagulopathy/hereditary hemorrhagic disorders.

    - vi. Neurologic disease.

- Participants will be excluded if they have taken the following, within the last 30 days or are unwilling to forgo the following for 30 days prior to entry into the study:

    - i. Calcium, Vitamin D, including multivitamins that have low amounts of calcium/Vitamin D, supplements with magnesium, and fiber supplements.

    - ii. Non-steroidal anti-inflammatory medications (NSAIDs), such as Naproxen or Ibuprofen (except for occasional pain control or low dose aspirin for cardiovascular disease prevention).

    - iii. Corticosteroids (a type of steroid drug such as prednisone or cortisol that helps your body to regulate your stress response, immune response and inflammation).

    - iv. Medications for GI symptoms daily such as 5-HT3 antagonists/5-HT4 agonists, prokinetic drugs, laxatives, anti-diarrheal, or antispasmodics.

- v. Medications that are known strongly affect the neuromodulation of pain of the GI tract, including: serotonin/catecholamines (SNRI-serotonin and norepinephrine reuptake inhibitors), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), first generation antipsychotics, and other specific atypical antidepressants.
    - SNRI examples are venlafaxine, desvenlafaxine, duloxetine, atomoxetine, sibutramine, and milnacipran
    - MAOI examples are selegiline, dicarboxamide, phenelzine, tranylcypromine
    - TCA examples are amitriptyline, imipramine, trimipramine, desipramine, nortriptyline, doxepin,
    - Excluded atypical antidepressants include mirtazapine, sibutramine, and bupropion
- vi. Medications that would serve as diuretics, including furosemide, hydrochlorothiazide, chlorothiazide, chlorthalidone, metolazone, spironolactone, indapamide, bumetanide, ethacrynic acid, torsemide, amiloride, eplerenone, and triamterene.
- vii. Medications that would drastically alter the intestinal microbiota, particularly antibiotics.

- Subjects must not have co-morbid pain or psychiatric conditions (e.g., fibromyalgia, bipolar or psychotic disorder).

<u>Inclusion Criteria for subjects with ulcerative colitis:</u>

- Must be able to give written informed consent.
- Male or female, ages 18 to 80 years old.
- Must have the following:
- Ulcerative colitis with confirmed diagnosis by histology and endoscopy; AND be in stable remission for 3 months or more without therapy or with a maintenance therapy (except steroids and antibiotics for 3 months) OR have mild ulcerative colitis. Note: Corticosteroids (a type of steroid drug such as prednisone or cortisol that helps the body to regulate stress response, immune response and inflammation) and antibiotics can be used during a flare-up once the study has begun and the subject enrolled.
- A negative pregnancy test for pre-menopausal women with intact female reproductive organs. The negative pregnancy test must be within 2 weeks of the baseline flexible sigmoidoscopy, and subject must agree to use appropriate birth control over the study period. Acceptable forms of birth control include: hormonal (such as pill, IUD), barrier (such as condom, diaphragm), surgical, or abstinence. Post-menopausal is defined as no menses for the previous 12 months. If cessation of menses is within 12 months, then the subject should be treated as pre-menopausal and a pregnancy test performed.

<u>Exclusion Criteria for subjects with ulcerative colitis:</u>

- Female subjects must not be pregnant or lactating; and female of childbearing potential unwilling to use acceptable birth control throughout the study.
- Must not be participating in any other interventional trial using an investigational drug.
- Subjects likely to be uncooperative or unable to comply with study procedures
- Participants must not be felt to have active ulcerative colitis for 3 months before study enrollment (with the exception of mild ulcerative colitis).
- Participants must not have a history or diagnosis of any of the following conditions:
    - Crohn's disease.
    - Any stomach or intestinal bleeding disorders (gastrointestinal bleeding from gastric or duodenal ulcers, or gastrin secreting tumors) or active gastric / duodenal ulcers - peptic ulcer disease (without bleeding in last 3 months).
    - Any gastrointestinal or colonic malignancy.
    - Kidney disease, including kidney "stones" or hypercalcemia.
- Participants will be excluded if they have taken the following, within the last 30 days or are unwilling to forgo the following for 30 days prior to entry into the study:
    - Calcium, Vitamin D, including multivitamins that have low amounts of calcium/Vitamin D and fiber supplements.
    - Non-steroidal anti-inflammatory medications (NSAIDs), such as Naproxen or Ibuprofen (except for occasional pain control or low dose aspirin for cardiovascular disease prevention). Note – IBD patients are cautioned to avoid NSAIDs in general since they are associated with flare of disease.

<u>Inclusion Criteria for Irritable Bowel Syndrome Type D Patients:</u>

- Must be able to give written informed consent.
- Patients with ages 18-80 years old.
- Must have the following to meet IBS-D Diagnostic Rome IV Criteria:
    - i. Recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with two or more of the following criteria:
        - a. Related to defecation
        - b. Associated with a change in frequency of stool (increased)
        - c. Associated with a change in form (appearance of stool)
    - ii. Symptom onset at least 6 months prior to diagnosis

- iii. Greater than twenty-five percent of bowel movements (BM) with Bristol Stool types 6 or 7 and less than twenty-five percent of bowel movements with Bristol stool types 1 or 2. BMs are usually diarrhea, “watery,” “liquid,” “fluffy pieces,” or “mushy”.

- A negative pregnancy test for pre-menopausal women with intact female reproductive organs. The negative pregnancy test must be within 2 weeks of the baseline visit and the subject must agree to use appropriate birth control over the study period. Acceptable forms of birth control include: hormonal (such as pill, IUD), barrier (such as condom, diaphragm), surgical, or abstinence. Post-menopausal is defined as no menses for the previous 12 months. If cessation of menses is within 12 months, then the subject should be treated as pre-menopausal and a pregnancy test performed.

- If currently on a mood medication, the subject must continue to have IBS symptoms like abdominal pain, bloating, bothersome constipation or diarrhea to be included. See below in exclusionary criteria for mood medications that would exclude participation in the study
  - the patients are allowed to be on the following medications throughout the study as long as the dosage and regimen is stable:
    - specific atypical antidepressants including trazodone, St john's wart
    - selective serotonin reuptake inhibitors including escitalopram, citalopram, fluoxetine, fluvoxamine, sertraline, paroxetine, and vortioxetine
    - second generation antipsychotics, including olanzapine, risperidone, and quetiapine

<u>Exclusion Criteria for Irritable Bowel Syndrome Type D Patients</u>:

- Must not be pregnant or lactating; patients of childbearing potential unwilling to use acceptable birth control throughout the study also excluded.

- Must not be participating in any other interventional trial using an investigational drug.

- Subjects likely to be uncooperative or unable to comply with study procedures

- Participants must not have a history or diagnosis of any of the following conditions:

  - i. Crohn’s disease or Inflammatory bowel disease.

  - ii. Any stomach or intestinal bleeding disorders (gastrointestinal bleeding from gastric or duodenal ulcers, or gastrin secreting tumors) or active gastric / duodenal ulcers - peptic ulcer disease (without bleeding in last 3 months).

  - iii. Any gastrointestinal or colonic malignancy.

  - iv. Kidney disease, including kidney “stones” or hypercalcemia.

  - v. Coagulopathy/hereditary hemorrhagic disorders.

  - vi. Neurologic disease.

- Participants will be excluded if they have taken the following, within the last 30 days or are unwilling to forgo the following for 30 days prior to entry into the study:

    - i. Calcium, Vitamin D, including multivitamins that have low amounts of calcium/Vitamin D, supplements with magnesium, and fiber supplements.

    - ii. Non-steroidal anti-inflammatory medications (NSAIDs), such as Naproxen or Ibuprofen (except for occasional pain control or low dose aspirin for cardiovascular disease prevention).

    - iii. Corticosteroids (a type of steroid drug such as prednisone or cortisol that helps your body to regulate your stress response, immune response and inflammation).

    - iv. Medications for GI symptoms daily such as 5-HT3 antagonists/5-HT4 agonists, prokinetic drugs, laxatives, anti-diarrheal, or antispasmodics.

    - v. Medications that are known strongly affect the neuromodulation of pain of the GI tract, including: serotonin/catecholamines (SNRI-serotonin and norepinephrine reuptake inhibitors), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), first generation antipsychotics, and other specific atypical antidepressants.
        - SNRI examples are venlafaxine, desvenlafaxine, duloxetine, atomoxetine, sibutramine, and milnacipran
        - MAOI examples are selegiline, dicarboxamide, phenelzine, tranylcypromine
        - TCA examples are amitriptyline, imipramine, trimipramine, desipramine, nortriptyline, doxepin,
        - Excluded atypical antidepressants include mirtazapine, sibutramine, and bupropion

    - vi. Medications that would serve as diuretics, including furosemide, hydrochlorothiazide, chlorothiazide, chlorthalidone, metolazone, spironolactone, indapamide, bumetanide, ethacrynic acid, torsemide, amiloride, eplerenone, and triamterene.

    - vii. Medications that would drastically alter the intestinal microbiota, particularly antibiotics.

- Subjects must not have co-morbid pain or psychiatric conditions (e.g., fibromyalgia, bipolar or psychotic disorder).

Research protocol: Each research subject will have 3 study visits. The scheduled events are presented in a Table below.

Visit #1: Screening visit. Interested, healthy subjects who responded to recruitment advertising and who qualify based on a phone/email screening questionnaire will be scheduled for a screening visit. At the screening visit, a member of the study team will describe the study and review the consent document. If the subject is eligible and interested in participating, they

will sign the informed consent form. The following tasks will be performed during the screening visit:

- Eligibility assessed
- Informed consent signed
- Questions asked about the concomitant medication/supplement use/medical history
- Medical history taken by an MCRU MA or study team member
- Vital signs assessed (seated blood pressure, pulse, and temperature)
- Wash out period established for steroids, antibiotics, NSAIDs and supplement use, if applicable
- Visit 2 (Baseline [Day 0] visit) scheduled
- Urine Pregnancy test performed (if applicable)
- Lactulose/mannitol (10 g lactulose and 5 g D-mannitol) powder kit with urine collection container, jug, and ice packs (if no wash out is required). This kit will be provided by UM Research Pharmacy.

Subjects will stop baby Aspirin (low dose) 7 days before the test and will not take any NSAIDs and steroids (these are already on the exclusion list). They will fast for 8 hours before the test. Subjects may eat and drink as usual the day before taking the test drink; however, they MUST avoid certain foods or beverages, especially those containing fructose, the day before (for 24 hours) except mannitol and marijuana-related products for 48 hours prior and during the test.

These include:

- Any marijuana product (for 48 hours before collection)
- Artificial sweeteners
- Fruits, fruit juices, jams, jellies, etc.
- Soft drinks (regular or diet sodas) and foods sweetened with high fructose corn syrup
- Alcoholic drinks
- Any dairy products
- Dietetic chocolate
- Honey
- Mushrooms, beans (legumes including peanuts), celery
- Chewing gum

<u>Visit #2: Baseline (day-0) visit</u>. Subjects will be instructed to fast overnight (8 hours) before the morning of the baseline visit. They will collect their first urine (pre-Lactulose/ Mannitol) sample (in the morning) according to the provided instructions and put it into the urine collection cup (container#1) kept on ice or refrigerated. After that, they will mix the lactulose/mannitol powder with 2 cups of water (8 oz. per cup) and drink it within 15-20 minutes. No food will be consumed during the first 8 hours of the entire urine collection period (24 hours) but a further 2 cups of water (8 oz each) will be given two hours after the ingestion of the sugar solution. For twenty-four hours after finishing the lactulose/mannitol, subjects will collect all of their urine and store it in the three jugs (container#2, 3 & 4) either refrigerated or on ice packs. Hence the total amount of fluid administered over an eight-hour period will be around 32 oz. They can resume their normal water and food intake after eight hours of collection except few items to avoid during the collection period. For the first 2 hours after finishing the lactulose/mannitol, subjects will collect all of their urine in container#2. For the next 6 hours (hours 2-8), urine will be collected in container#3. Both containers or jugs (container#2 & 3) either be refrigerated or stored on ice packs. From hours 8-24, subjects will collect their urine and store it in a jug (container #4) either refrigerated or on ice packs. Subjects will be allowed to eat non-fructose containing foods between hours 8 to hour 24, but must document fluid intake

given anticipated variability. Please see fluid intake form for how this will be documented by study subjects. This regime facilitates a twenty-four-hour urine sample collection, brought by the subject. Permeability will be assessed (in the lab) as the lactulose:mannitol ratio using the *BioAssay Systems EnzyChrom Assay Kit.* Additional samples will be saved for sucrolase assessment. However, we do not plan to assess sucrolase unless there is no evidence of permeability change based on the lactulose:mannitol ratio.

When subjects arrive at the MCRU, they will bring their urine samples with them. Vital signs will be assessed (as in Visit #1). Morphometric measurements (height, weight, and waist/hip measurements) will be collected. Urine Pregnancy test will be performed (if applicable). Subjects will again have the study purpose and methods explained. At that point, they may resume normal food and water consumption. The blood will be drawn and analyzed per metabolic panel test. In addition, a blood sample will be collected and saved for future research purposes, e.g., to conduct barrier permeability assay. The quantity of blood collected will be equivalent to about three teaspoons or 14 ml at each blood collection event. For subjects with UC, C-Reactive Protein (CRP) in blood will be assessed. Subjects with UC will also answer a disease-related questionnaire known as Inflammatory Bowel Disease Questionnaire (IBDQ). Subjects with IBS-D will answer a disease related questionnaire known as, the IBS quality of life questionnaire (IBS-QOL).

After the completion of the 24-hour urine collection, subjects will be given a 90-day supply of Aquamin® along with instructions for taking the agent. Dietary History questionnaire will be administered past year food intake. After 90 days on the intervention, subjects will return to the MCRU for Study Visit #3 (final visit). Visit #3 will be conducted in a manner similar to that of Visit #2. A payment of $20.00 for completion of Visit #2 will be arranged.

Monthly monitoring: Day 30 and 60 (± 5 days), assessed toxicity, adherence and reminded about diet health questionnaire by telephone or email.

Study Visit #3 (day-90) (Endpoint Visit): On Day 90 (± 5 days), subjects will be asked the same questions as at Visit #2. In addition, they will be asked questions about their experience with the study agents and, in particular, if there were any tolerability issues or safety concerns.

Subjects will be instructed to fast overnight (8 hours) before the morning of the final visit. They will collect their first urine sample (in the morning) prior to lactulose/mannitol intake according to the provided instructions and put it into container#1 (provided on ice packs). After that, they will mix the lactulose/mannitol powder with 2 cups of water (8 oz) and drink it within 15 minutes. No food will be consumed during the first 8-hour segment of the entire urine collection period (24 hours) but an additional 2 cups of water (8 oz. each) will be given two hours after the ingestion of the sugar solution. Hence, the total amount of fluid administered over an eight-hour period will be around 32 oz. For the first 2 hours after finishing the lactulose/mannitol, subjects will collect all of their urine in container#2. For the next 6 hours (hours 2-8), urine will be collected in container#3. Both containers or jugs (container#2 & 3) either be refrigerated or stored on ice packs. They can resume their normal water and food intake after eight hours of collection except few items to avoid during the collection period. From hours 8-24, subjects will collect their urine and store it in a jug (container #4) either refrigerated or on ice packs. Subjects will be allowed to eat non-fructose containing foods between hours 8 to hour 24, but must document fluid intake given anticipated variability. This regime facilitates a twenty-four-hour urine sample collection, brought by the subject. Permeability will be assessed (in the lab) as the lactulose:mannitol ratio using the *BioAssay Systems EnzyChrom Assay Kit.* Additional samples will be saved for sucrolase assessment. However, we do not plan to assess

sucrolase unless there is no evidence of permeability change based on the lactulose:mannitol ratio.

When subjects arrive at the MCRU, they will bring their urine samples with them. Vital signs will be assessed (as in Visit #1). Morphometric measurements (weight and waist/hip measurements) will be collected. Subjects will again have the study purpose and methods explained. At that point, they may resume normal food and water consumption. The blood will be drawn and analyzed per metabolic panel test. In addition, a blood sample will be collected and saved for future research purposes, e.g., to conduct barrier permeability assay. For subjects with UC, C-Reactive Protein (CRP) in blood will be assessed. Subjects with UC will also answer a disease related questionnaire known as Inflammatory Bowel Disease Questionnaire (IBDQ). Subjects with IBS-D will answer a disease-related questionnaire known as, the IBS quality of life questionnaire (IBS-QOL).

The completion of Visit #3 will conclude the study participation and subject will return all the unused capsules with the capsule log, and disease-related survey (UC or IBS-D) and Dietary History Questionnaire collected. A payment of $40.00 for completion of Visit 3 will be arranged.

Lactulose:mannitol ratio to assess gastrointestinal permeability. When all of the samples from a given subject have been collected, the samples will be assayed in one batch. The *BioAssay Systems EnzyChrom Assay Kit* will be used for this.

Schedule of Events.

Evaluation/Procedures	Screening-visit 1	Baseline-visit 2 Day 0	[A] Monthly Monitoring	Final-visit 3 Day 90±5
Informed Consent	X			
Medical History	X			X
Morphometric measurements [B]		X		X
Urine Pregnancy Test [C]	X	X		
Concomitant Medication/ Supplement Use/ normal diet	X	X	X	X
Disease assessment by IBDQ (UC subjects only) [D]		X		X
Disease assessment by IBS-QOL (IBS-D subjects only) [D]		X		X
Drug assignment (Single group)		X		
Dispense Lactulose/Mannitol	X	X		
Dispense Study Medication		X		
Adherence/Toxicity Evaluation/ Adverse Events		X	X	X
Dietary History Questionnaire [E]		X	X	X
Off-Study Form				X
Pill Count				X
Blood draw [F]		X		X
Urine collection (for Lactulose:Mannitol ratio) / Fluid intake form		X		X

[A] Monitoring (for assessment of toxicity, adherence and a reminder to complete the diet questionnaire) will be completed by phone call or email reminder on the following days; Days 30, and 60 (+/- 5 days).

[B] Morphometric measurements will consist of body weight, height, and waist/hip measurements. Height will be measured only on Visit 2.

[C] The pregnancy test which must be valid within 14 days of the first dose of study medication. If the time between visit 1 and visit 2 is greater than 14 days, the pregnancy test may be done at visit 2 only or be repeated.

[D] IBDQ or IBS-QOL will be offered electronically and should be completed on Days 0, and 90 (+/- 5 days).
[E] Given at visit 2 and collected at the visit 3 with reminders at phone monitoring.
[F] For clinical and research purposes (Research sample will be saved in a "Red Top Vacutainer® Tube).

Statistical design and data handling: At the end of the study, we will have lactulose:mannitol ratios (a single value) from each subject at each time-point: Visit #2, and Visit #3. Change in ratio between visits will be plotted for each subject separately so that trends in a given subject can be detected. Following this, ratios from all 12 subjects in each cohort separately) will be averaged from each visit. Means and standard deviations will be determined and values from each visit will be compared using ANOVA followed by paired sample comparison. Finally, we will compare the three cohorts to one another in order to determine if differences between healthy subjects, those with UC, and those with IBS-D can be detected prior to intervention and to determine if intervention influences values in one cohort more than the other.

Relationship to main study: Other events. In other respects, subject interactions will be similar to those in the parent study. See Sections 8 (Adverse Events), 9 (Stopping Rules) and 10 (Data Management) from the parent study.

COVID-19 contingency plan: If final visits are delayed due to COVID-19 circumstances, the research pharmacy may dispense an additional 30-day supply of capsules, up to three times, and the study team may collect additional safety measurements (for example, assessments of AEs via phone).

CITATIONS

1. Coskun M. Intestinal epithelium in inflammatory bowel disease. Front Med (Lausanne). 2014;1:24. doi: 10.3389/fmed.2014.00024. PubMed PMID: 25593900; PubMed Central PMCID: PMCPMC4292184.
2. Salim SaY, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. Inflammatory bowel diseases. 2011;17(1):362-81.
3. Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. World journal of gastroenterology: WJG. 2014;20(5):1165.
4. Vivinus-Nebot M, Frin-Mathy G, Bzioueche H, Dainese R, Bernard G, Anty R, et al. Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. Gut. 2014;63(5):744-52.
5. Lee JY, Wasinger VC, Yau YY, Chuang E, Yajnik V, Leong RW. Molecular pathophysiology of epithelial barrier dysfunction in inflammatory bowel diseases. Proteomes. 2018;6(2):17.
6. Holtmann GJ, Ford AC, Talley NJ. Pathophysiology of irritable bowel syndrome. Lancet Gastroenterol Hepatol. 2016 Oct;1(2):133-146. doi: 10.1016/S2468-1253(16)30023-1. Epub 2016 Sep 8. PMID: 28404070.
7. Marshall JK, Thabane M, Garg AX, et al. Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. Aliment Pharmacol Ther 2004; 20: 1317–22.
8. Vazquez-Roque MI, Camilleri M, Smyrk T, et al. Association of HLA-DQ gene with bowel transit, barrier function, and inflammation in irritable bowel syndrome with diarrhea. Am J Physiol Gastrointest Liver Physiol 2012; 303: G1262–69.
9. Shulman RJ, Jarrett ME, Cain KC, Broussard EK, Heitkemper MM. Associations among gut permeability, inflammatory markers, and symptoms in patients with irritable bowel syndrome. J Gastroenterol 2014; 49: 1467–76.
10. Annaházi A, Gecse K, Dabek M, Ait-Belgnaoui A, Rosztóczy A, Róka R, Molnár T, Theodorou V, Wittmann T, Bueno L, Eutamene H. Fecal proteases from diarrheic-IBS and ulcerative colitis patients exert opposite effect on visceral sensitivity in mice. Pain. 2009

Jul;144(1-2):209-17. doi: 10.1016/j.pain.2009.04.017. Epub 2009 May 17. PMID: 19450926. (citation 1 for protease and visceral sensitivity)

11. Gecse K, Róka R, Ferrier L, Leveque M, Eutamene H, Cartier C, Ait-Belgnaoui A, Rosztóczy A, Izbéki F, Fioramonti J, Wittmann T, Bueno L. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. Gut. 2008 May;57(5):591-9. doi: 10.1136/gut.2007.140210. Epub 2008 Jan 14. PMID: 18194983. (citation 2 for protease and visceral sensitivity)
12. Bertiaux-Vandaële N, Youmba SB, Belmonte L, et al. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. Am J Gastroenterol 2011; 106: 2165–73.
13. Wilcz-Villega EM, McClean S, O'Sullivan MA. Mast cell tryptase reduces junctional adhesion molecule-A (JAM-A) expression in intestinal epithelial cells: implications for the mechanisms of barrier dysfunction in irritable bowel syndrome. Am J Gastroenterol 2013; 108: 1140–51.
14. Martínez C, Lobo B, Pigrau M, et al. Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. Gut 2013; 62: 1160–68.
15. Vivinus-Nébot M, Dainese R, Anty R, Saint-Paul MC, Nano JL, Gonthier N, Marjoux S, Frin-Mathy G, Bernard G, Hébuterne X, Tran A, Theodorou V, Piche T. Combination of allergic factors can worsen diarrheic irritable bowel syndrome: role of barrier defects and mast cells. Am J Gastroenterol. 2012;107(1):75-81. doi: 10.1038/ajg.2011.315.
16. Attili D, McClintock SD, Rizvi AH, Pandya S, Rehman H, Nadeem DM, Richter A, Thomas D, Dame MK, Turgeon DK, Varani J, Aslam MN: Calcium-induced differentiation in normal human colonoid cultures: Cell-cell / cell-matrix adhesion, barrier formation and tissue integrity. PLoS One 14(4): e0215122, 2019. PM30995271
17. Aslam MN, McClintock SD, Attili D, Pandya S, Rehman H, Nadeem DM, Jawad-Makki MA, Rizvi AH, Berner MM, Dame MK, Turgeon DK. Ulcerative Colitis-Derived Colonoid Culture: A Multi-Mineral-Approach to Improve Barrier Protein Expression. medRxiv 2019.12.12.19014662; doi: https://doi.org/10.1101/2019.12.12.19014662
18. McClintock SD, Attili D, Dame M, Richter A, Silvestri SS, Berner MM et al. (2020) Differentiation of human colon tissue in culture: Effects of calcium on trans-epithelial electrical resistance and tissue cohesive properties. PloS One. 15(3): e022058.
19. Aslam MN, Bassis CM, Bergin IL, et al. A Calcium-Rich Multimineral Intervention to Modulate Colonic Microbial Communities and Metabolomic Profiles in Humans: Results from a 90-Day Trial. Cancer Prev Res (Phila). 2020;13(1):101–116.
20. Aslam MN, McClintock SD, Knuver K, Jawad-Makki MAH, Ahmad H, Turgeon DK, Varani J: A multimineral approach to alter biomarkers of growth and differentiation in the human colon: Results from an interventional trial (CT-112 Clinical Trial Abstract - Oral presentation), American Association for Cancer Research (AACR) Virtual Annual Meeting 2020, Virtual, Abstract # 10533, 2020.
21. Aslam MN, McClintock SD, Jawad-Makki MAH, Knuver K, Ahmad HM, Basrur V, Bergin IL, Zick SM, Sen A, Turgeon DK, Varani J. A Multi-Mineral Intervention to Modulate Colonic Mucosal Protein Profile: Results from a 90-Day Trial in Human Subjects. Nutrients. 2021; 13(3):939. https://doi.org/10.3390/nu13030939.
22. Sequeira IR, Lentle RG, Kruger MC, Hurst RD. (2014) Standardising the Lactulose Mannitol Test of Gut Permeability to Minimise Error and Promote Comparability. PLoS One 9(6): e99256.