"Human pilot study - HA35 dietary supplement for promoting intestinal health"

Detailed Protocol

Twenty healthy adult volunteers, ten with a lean BMI between 19-25 and ten with an obese BMI between 30-35, will complete this study. There will be no restriction to gender or race. Age limits will be 18-45 years for this pilot study, since ageing may affect intestinal immunity, microbiome composition, prevalence of pre-clinical disease confounders[1],[2]. The screening visit will include medical history, physical examination, diet history (including probiotics and food allergies) using the Diet History Questionnaire II [3], and laboratory testing (liver function testing, basic metabolic panel, complete blood count, indirect calorimetry). Once entered to the study, subjects will be asked to refrain from chronic NSAIDs (i.e. more frequent dosing than twice per week) and acid suppressants, and notify the study team if they are prescribed new systemic (oral, intravenous) or local enteric (laxatives, enemas) medications. Starting at D1, subjects will ingest 140 mg of HA35 dissolved in purified sterile water that is provided in individual pre-made doses (supplied by the CRU team) once daily, 30 min. before breakfast. The initial dosage was chosen from the functional dose we determined in mice and using intestinal surface area ratio calculations[4] scaled to an average 70k human, and multiplied by 2 for the maximal potential weight of a study participant. The dosage derived by this method is slightly higher, but still in general agreement with the calculated dose recommended by body surface area conversion methods[5]. The seven HA35 doses will be made in the kitchen of the Clinical Research Center just prior to the start of each volunteer's treatment course.

Study subjects will be asked to keep a daily log of gastrointestinal symptoms, a food diary, and a stool frequency chart[6]. At days D-0, D-7 and D-28 subjects will provide a stool sample from the entire day. If subjects do not have daily bowel movements they can provide a stool sample on D-1 or D0, D7 or D8, and D27 or D28. Stool samples will be refrigerated immediately after collecting and stored long-term at -80° C until analyzed (samples will be homogenized before sampling) for microbiome and antimicrobial peptide determination. Additionally subjects will provide blood samples at the same time points for analysis as outlined in primary and secondary outcomes (see below). Subjects will be seen at the Clinical research Unit at the Cleveland Clinic at D0, D8 and D28 of the study and donate samples.

Inclusion criteria:

- BMI 19-25 (lean), and BMI 30-35 (obese)
- Age 18-45 years old
- Willingness to take oral supplement and adhere to study requirements

Exclusion criteria:

- Diabetes

- Oral antibiotics within 4 weeks of study initiation
- History of cardiac disease, and medications for cardiac disease
- Use statins and antihypertensive drugs
- Inflammatory bowel disease including irritable bowel syndrome
- History of intestinal surgery, excluding hernia repair and appendectomy
- Active cancer diagnosis (except skin cancer)
- Chronic acid suppression treatment (proton pump inhibitors, histamine H2 receptor antagonists)
- Immune modulatory treatments (e.g. chronic immunosuppressive medications, chronic NSAIDs)
- Vegetarian or vegan diet[7]
- Abnormal liver or kidney function as measured by routine serum chemistry testing
- Severe anemia or significant white blood cell or platelet abnormalities
- No additional blood or blood product donations during the study

Objectives

- To evaluate whether oral HA35 supplementation modifies the human gut microbiome
- To confirm the safety profile of oral HA35 treatment
- To evaluate whether oral HA35 increases intestinal defensin levels, and decreases intestinal inflammation and permeability
- To assess the health benefits that may result from oral HA supplementation (improved BMI, serum panels)

Study Endpoints

Study outcomes include: **1**) stool microbiome diversity and phylogenetic distribution (primary); **2**) assessment of comprehensive metabolic panel (CMP), complete blood count (CBC); **3)** indirect calorimetry measurements using the respiratory quotient (RQ); **4**) indices of fecal intestinal antimicrobial peptide secretion (calprotectin, human beta-defensin; **5**) serum indicators of intestinal permeability (serum HA, serum LPS, serum I-FABP); **6**) serum indicators of inflammation and injury (high-sensitivity C-Reactive Protein, IL6 and TNFα). Oral HA35 supplementation may prove to have a prebiotic effect and modulate intestinal as well as systemic inflammation through a modulation of the intestinal microbiome and epithelial defense system.

Methods

Composition of stool microbiome as measured by 16S pyrosequencing will beconducted by Second Genome on a fee for sample basis. The CMP and CBC will be performed by the Cleveland Clinic Department of Laboratory Medicine (fee/ sample). Indirect calorimetry measurement to determine changes in resting energy expenditure will be conducted in the CRU using Vmax Encore sampler and Care Fusion conversion software[8]. Stool calprotectin levels, stool HBD2 levels, serum HA, serum I-FABP, serum high-sensitivity CRP and serum LPS (ELISA) will be determined using commercially available ELISA kits and performed in the de la Motte laboratory.

Statistical analysis

We have calculated that we need at least 20 subjects, and up to 30 subjects, providing several samples to be able to detect an effect of our intervention on the microbiome primary endpoint, i.e. microbial diversity and phylogenetic distribution. This is in agreement with interventional studies investigating the modification of intestinal microbiome in obese individuals that have analyzed between 9 and 28 subjects[7,9,10]. We propose to recruit 20 subjects in order to ensure sufficient power.

Safety

Serpil Erzurum, MD, director of the Cleveland Clinic Clinical Research Unit, is the safety and emergency contact physician for this study. Dr. Erzurum and can be reached at

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Section 6 references:

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