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Carrageenan in ulcerative colitis

Study protocol, prepared by Reijo Laatikainen, Booston Oy, tel. +358 40 7171 753, reijo.laatikainen@booston.fi

Introduction

Incidence of inflammatory bowel disease (IBD) is increasing in Western countries, affecting approximately 0.7 % of different populations in Western countries. Increase in the incidence of IBD has been particularly high in Finland and Nordic countries[1]. Dietary factors, along with other environmental factors, are postulated to play role in this development[2]. Increased consumption of processed foods, low intake of fiber and overconsumption of red and processed meat, all represent typical major shifts in dietary practices in many Western populations[2,3,4]. In recent clinical studies, neither reduction of red meat consumption, nor fiber supplements, have shown promise in the prevention of colitis in people with IBD[3,4]. The question whether food processing, especially food additives, play a crucial role in the development of colitis, is largely unknown – therefore, there is an urgent need for randomized studies in this area of research[5].

Carrageenan is a red seaweed-based emulsifier broadly used by food industry[6]. It is sulfated polygalactan. High molecular carrageenan (>200 000 daltons) is also called food-grade carrageenan. It is commonly used in puddings, milk, soy and oat based drinks/creams, yogurt, low-fat ice cream, jellies, marmelade and processed meat[6]. Its global use is increasing[7].

Consumption of carrageenan in EU ranged from mean 35 mg/kg (body weight) per day in adults to mean 139 mg/kg per day according to EFSA (European Food Safety Association)[8]; this calculation is based on so called maximum level exposure assessment scenario. In populations which use very little milk-based processed foods mean carrageenan consumption can be as low as 5 mg/kg. It is noteworthy that toddlers seem to consume 3-5 times more carrageenan per kilogram[8].

Carrageenan induces intestinal hyperpermeability in vitro studies (Caco-2 model), is proinflammatory in animals and causes disturbances in gut microbiota of animals[9]. Actually, low molecular weight carrageenan, also called as poligeenan (<20 000 daltons) is used to cause experimental colitis in animal models of ulcerative colitis[9]. It is postulated that only low-molecular carrageenan causes intestinal inflammation, and that food-grade carrageenan is neutral in this respect. However, effects of food-grade carrageenan on gastrointestinal symptoms, inflammatory and permeability markers have not been studied in placebo-controlled randomized controlled

trials[6]. One small randomized study with major limitations suggested that carrageenan might be proinflammatory in such context among people with ulcerative colitis[10]. These preliminary results have not been confirmed by other research groups.

2. Aim

To explore if food-grade carrageenan causes gastrointestinal symptoms, inflammation and hyperpermeability in patients with ulcerative colitis when consumed at high normal level according to EU standard population.

3. Methods and material

3.1 Sample size, recruiting and inclusion criteria

Subjects aged 18-64 years will be recruited via advertisements in Facebook and the internet (e.g. www.pronutritionis.net, www.tervevatsa.fi). The main inclusion criterium is previously diagnosed ulcerative colitis in remission. Patients are treated by either life style, 5-ASA or thiopurine derivatives such as azathioprine. Further inclusion criteria include willingness to consume carrageenan and beta-glucan (placebo) for 7-day treatment periods. Subjects are excluded if they have relapse (i.e. active disease phase) of ulcerative colitis, use of biologic treatment modalities or corticosteroids, intestinal surgery, cancer or other severe illness which might affect patients' ability to participate in the study. Also, pregnant and lactating women, alcoholics or subjects taking medications potentially influencing gastrointestinal function and subjects participating in any other clinical trial are not eligible.

A total of 16 subjects will be recruited. The sample size calculation was based on the primary outcome measure Simple Clinical Colitis Assessment Index (SCCAI)[11]. Suitable previously published data were not available to be used in power calculations. Therefore, we assumed that the difference between study products would be at least 2 points on the 19-point SCCAI score and that the standard deviation of that difference would be 1.5 points. Thus, a sample size of 14 would have 90% power to detect this 2-point

difference when using a paired t-test with a 0.05 two-sided significance level. The anticipated drop out was 10–20% and therefore 16 patients are targeted for this cross-over study.

Pre-screening of candidates are done in a telephone interview. Subjects meeting preliminary inclusion criteria were invited to a screening study visit, where their health status, disease activity (remission/relapse), possible medications and dietary restrictions are to be evaluated. Before entering the study, all subjects are to provide written informed consent.

3.2. Withdrawal from the study

Participants have right to withdraw from the study at any time without an obligation to give reasons for the discontinuation. Information about right to withdraw is given to all participants before entering the study both verbally and in written.

3.3. Screening visit

All eligible participants are asked to participate screening visit. During the visit all possible diseases and medications, height, weight and sex are enquired by using the survey (Appendix Background infromation). The nature and the course of the study is explained in detail. The eligible participants are informed via email and first research visit is agreed upon.

3.4. Course of research

This is randomised cross-over study where each participant consumes 7 days of both carrageenan and placebo. A wash out period of minimum 14 days separates the periods from each other. Randomisation is performed in blocks of four by automated programme. A person who was not involved with enrolling the participants or assigning them to the interventions will generate the randomisation list. Both the investigators and the subjects are to remain blinded to the randomisation. The study products are given at the beginning of each treatment period. Furthermore, all participants are instructed to follow otherwise carrageenan free diet by the study dietitian. The study design is illustrated in the Figure 1.

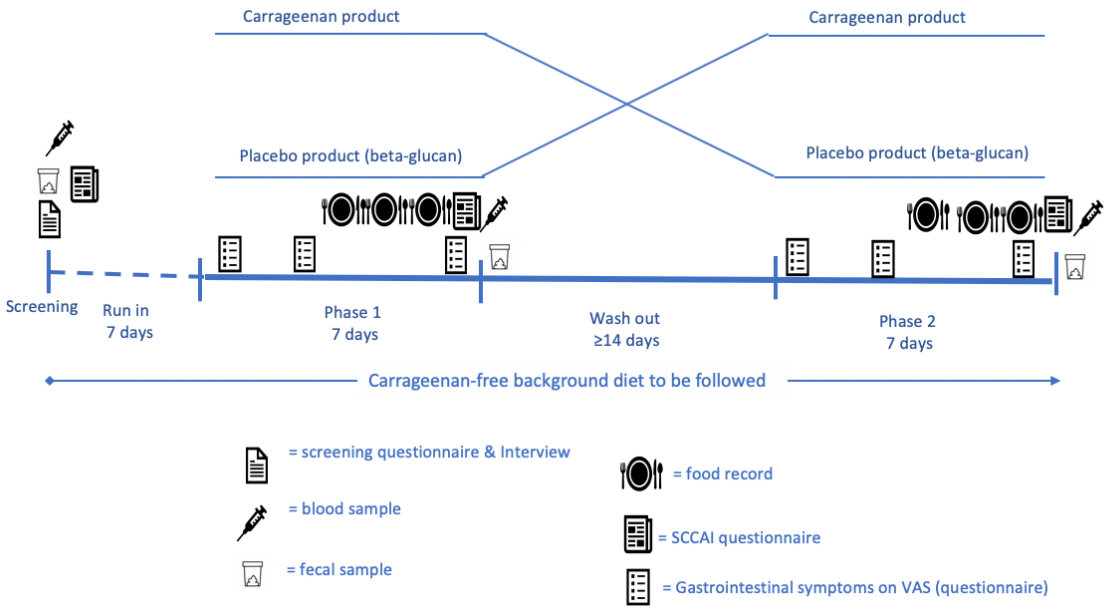

Figure 1. Study design

3.5. Research visits

All participants visit the research center at the beginning of each periods, i.e. 3 times. Visit are organized at Selexlab Oy, Helsinki, Kalevankatu 17 A, 00100 Helsinki. Blood samples are taken on each visit and SCCAI-questions are interviewed by the study dietician or nurse, and the use of VAS-symptom questionaires is instructed and delivered to the participants. Fecal samples are taken at home in the morning of the visit day, and given to researchers when checking in to the visit.

3.6. Disease activity, symptom assessment and food diaries

SCCAI Score (Simple Clinical Colitis Activity Instrument) is used for primary outcome. It is validated questionnaire to assess the activity of inflammatory bowel disease during the last seven days[11,12]; the questionnaire is filled by the research physician who interviews the participants covering all the SCCAI questions. The Finnish version of SCCAI questionnaire is the appendix SCCAI.
A total of 10 gastrointestinal symptoms are recorded (VAS 0–100 mm for each) at the baseline (screening visit) and at day 1, 3 and 7 of both study periods, i.e. 3 times. VAS questionnaire in

Finnish can be found in the appendix VAS. Food diaries are filled for 3 last days during the two treatment periods. Finnish food diary is in the appendix Food diary.

3.7. Blood,urine and fecal samples

Blood samples (20 ml) are drawn from the vein at the inside of elbow, three times as depicted in the figure 1. Hs-CRP (inflammatory marker), creatinine and FABP-2 (permeability marker) are determined from the blood samples, and these will be analyzed at Folkhälsan laboratory by the collaborator party of the study. Fecal samples are taken at home from the first gut passage of the day; a small container (approximately 20 ml) with special spoon is used. Participants bring the fecal samples with them to the researchers. Fecal calprotectin (inflammatory marker), fecal intestinal alkaline phosphatase (IAP) activity, and levels of fecal immunoglobulins (IgG, IgM, IgA) will be determined from the fecal samples. Overnight urine collection is done at the same timepoints as fecal sample collection: albumin and creatinine is determined in this samples.

3.8. Background diet, sleep and exercise

All participants are instructed follow carrageenan free diet. In practice, puddings, flavored yogurts and oat/soy yogurts, whip cream, long-self life cream, oat crème fraiche, milk shakes, vanilla and chocolate sauces, low-fat ice cream, convenience foods, marmalade and sausages and cold cuts are to be avoided during all study periods, including run-in and washout period. The study dietician instructs carrageenan-free diet to all participants. Participants are encouraged to maintain their current level of exercise and sleeping habits. Heavy drinking, more than 2 standard doses of alcohol/day, is discouraged throughout the study.

3.9. Carrageenan and placebo products

Carrageenan is provided to the participants as powder to be dissolved with heated oat based drink (Oddly Good Barista). Oat drink based chocolate shake is prepared at homes of each participant so that 2 deciliters of the oat drink contains 1000 mg carrageenan and needed amount of sucrose-sweetened chocolate powder; it estimated that 20 grams of cocoa powder is needed/day. Each participant needs to drink 4.0 deciliters of the shake/day in order to achieve targeted intake (2000 mg) of carrageenan solely from the study product. Placebo drink is composed in a similar manner, only difference being that carrageenan is replaced by oat fiber (Voimakaura, runsaskuituinen, Fazer

ltd). Oat, gluten free grain, is usually well tolerated by the patients with inflammatory bowel disease and other gastrointestinal diseases/disorders[13]. Furthermore, beta-glucan is considered as neutral or even mildly anti-inflammatory on the basis of preliminary studies [14,15]. Beta-glucan also forms gel when heated and mixed with water and thus resembles carrageenan from the physio-rheological properties; consequently, oat fiber is reasonably well suited for serving as placebo. Both products are packed in to small identical transparent plastic pouches, and cocoa powder is provided for free.

3.10. Adverse events

Possible adverse events are followed during the whole study period and reported by using the adverse event formulary (Appendix Adverse event).

4. Timetable

Recruitment period is planned to be the second/third quarter of 2021 and the actual study is aimed to be performed between late 2021 and early 2022. Analysis of biological samples and symptom assessment is targeted to be executed during the first quartal 2022. Recruiting will be initiated only when ethical application has been approved by Helsinki University Hospital ethical committee.

5. Data management, privacy and statistical analyses

All participants are informed at the beginning of the study what data is gathered regarding them and how these data are handled. All gathered data is confidential by nature and will not be accessed by anyone else but the researchers themselves. All personal information regarding symptoms, biochemical measurements, food data and background information is depersonalized by secret coding system so that any kind of identifiers, such as name, social security numbers, are absent. Gathered research data is kept in a locked room at the premises of Booston Oy, Viikinkaari 6, Helsinki. If a volunteer is deemed to be unsuitable for the study at the screening phase, her/his data are deleted. Electrical data are saved in Booston's database, which is protected by user name and pass code. The personal information of each participant can only be attained by using a secret code which is kept at premises of Folkhälsan, Helsinki. The study report will not disclose personality of any individual in the study. No participant information is gathered from patient

database of any public hospital or registry. Research data is kept for 10 years in a locked room of Booston premises and thereafter will be destroyed.

Forms to be used in the study

- Personal and background information (Appendix Background information)
- Research information and timetable (Appendix Overall information and timetable)
- Screening forms (Appendix Screening)
- SCCAI form (Appendix SCCAI)
- Gastrointestinal symptoms, VAS form (Appendix VAS)
- Food diary (Appendix Food Diary)
- Adverse event form (Appendix Adverse Event)
- Discontinuation form (Appendix Discontinuation)
- Night urine collection (Appendix Urine Collection)

6. Statistical analyses

Disease activity assessed by SCCAI is the primary end point of the study. VAS based gastrointestinal symptoms and biochemical measurements are secondary outcomes. Outcomes measures are analyzed by using primarily intention to treat (ITT) principle. Variance analysis for cross-over study design is used. Potential carry over effect is analyzed.

7. Ethical considerations

Participants are recruited via internet (Reijo Laatikainen's sites: pronutritionist.net, tervevatsa.fi and at the patient association Crohn&Colitis) by using the attached recruitment advertisement (Appendix Recruitment). All participant have a right to withdraw from the study at any point. Before the initiation of the study participants are informed about the study both in written and verbally and they also sign written consent prior entering the study (Appendix Consent). This study is not directed, and thus not accept under-

aged (<18 years), or people at retirement age (>65 years), pregnant, lactating, imprisoned or handicapped persons into the study. The research is carried out according to the Declaration of Helsinki, good clinical practice and prevailing regulations. Approval of ethical committee at HUS is applied. The study will be pre-registered into a relevant database, such as ISRCTN registry.

Carrageenan is widely available food additive in European food chain. It is approved as safe for use in a population by European Food Safety Authority (EFSA) and American Food and Drug Authority. Overwhelming majority of population is exposed to it regularly. Therefore, there is no reason to expect any serious adverse events to occur. If a participant may experience anything that requires medical attention she/he is directed to further investigations and medical treatment. Drawing of blood samples as well as handling of own fecal samples may feel unpleasant. Participating the study does not entail any financial or any direct benefit for the participants. All participants are informed about the personal results regarding biochemical measurements and symptom assessments. At the end of study, all participants are allowed to consult (45 min) the study dietician for diet related matters for free of charge.

8 Research organisation and division of work (in Finnish)

Nimi	Rooli	Akateeminen arvo / tutkinto	Terveydenhuollon ammattihenkilö	Työnantaja	Sähköposti
Hillilä Markku	Tutkimuksesta vastaava henkilö (TVH)	Tohtori	Kyllä	HUS	markku.hillila@hus.fi
Laatikainen Reijo	Vastuu-, yhteyshenkilö ja toimeksiantaja	Tohtori	Kyllä	Booston Oy	reijo.laatikainen@booston.fi
von Behr Anneli	Tutkimusryhmän jäsen	Laborantti	Kyllä	Eläkkeellä	anneli.vonbehr@gmail.com
Turpeinen Anu	Tutkimusryhmän jäsen	Tohtori	Ei	Valio	anu.turpeinen@valio.fi
Lehto Markku	Tutkimusryhmän jäsen	Tohtori	Ei	Folkhälan	markku.lehto@helsinki.fi
Salmenkari Hanne	Tutkimusryhmän jäsen	Tohtori	Ei	Folkhälsan	hanne.salmenkari@helsinki.fi
Sontag-Strohm Tuula	Tutkimusryhmän jäsen	Tohtori	Ei	HY	tuula.sontag-strohm@helsinki.fi
Mäkelä Noora	Tutkimusryhmän jäsen	Tohtori	Ei	HY	noora.makela@helsinki.fi

9. Funding and insurances

Research costs are covered by personal grants from Juhani Aho medical research fund and by Booston oy Ltd. Carrageenan and Oat drink is provided by Valio Ltd and beta-glucan (oat fiber) is manufacter by Fazer Ltd and bought at supermarkets in Finland. Participants are not paid for participation. Booston Oy has

patient insurance which covers potential costs to participant according to the patient injury law, in case of study-related patient injury.

10. Research report

The results will be published. A research article will be submitted to relevant scientific jounal in the field of gastroenterology or nutrition.

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