A randomized, placebo-controlled, double blind volunteer study into the effect of milk ingredients on gastroenteritis caused by an attenuated E. coli.

Published: 19-11-2012
Last updated: 26-04-2024

We want to determine whether Lacprodan® PL-20 can effectively protect against enterotoxigenic E.coli induced travellers* diarrhea in humans. In the present double-blind, placebo-controlled, randomized parallel study, the effect of oral milk protein...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Gastrointestinal infections
Study type	Interventional

Summary

ID

NL-OMON37279

Source
ToetsingOnline

Brief title
MIRAGE

Condition

- Gastrointestinal infections
- Bacterial infectious disorders

Synonym
gastroenteritis, traveller's diarrhea

Research involving
Human

Sponsors and support

Primary sponsor: Arla Foods Ingredients Group P/S
Source(s) of monetary or material Support: Arla Foods Ingredients Group P/S

Intervention

Keyword: E. coli, infection, milk, oral vaccin

Outcome measures

Primary outcome

Primary study outcomes

•Fecal ETEC excretion with time as marker of the colonization resistance

•Total daily fecal output as marker of diarrhea

Secondary outcome

Secondary study outcomes

•Bowel habits

•Frequency and severity of gastrointestinal symptoms

•Diarrhea severity (as measured by fecal dry weight excretion and % fecal dry weight)

•Specific serum antibody response to CFA-II

Tertiary study outcomes

•Opportunistic pathogens in feces

•Calprotectin in feces

•Total faecal and salivary sIgA

The performance of analyses of tertiary study outcomes will depend on the results of the primary and secondary study outcomes.

Study description

Background summary

Food-borne infections
Food-borne infections occur frequently. The WHO reported in 2007 that in industrialized countries, the percentage of the population suffering from food-borne diseases each year is up to 30%. This is probably an underestimation, since recent data from a Dutch study indicates that the incidence of infectious intestinal disease is 964 per 1000 person years 3. Food-borne infections are also frequently encountered by travelers to tropical countries, with incidences up to 80%. After some days of diarrhea, stomach pain, nausea or vomiting, most infections are self-limited and cured. However, such ordinary infections can be life-threatening in people with reduced resistance (e.g. young children, elderly, or persons taking immune-suppressive drugs). Treatment of food-borne infections with antibiotics is usually non-effective. Moreover, many bacterial pathogens become resistant to these drugs. Therefore, it is important to search for alternative means to prevent or treat these infections.

Milk ingredients
Enhancement of human resistance to food-borne infections is an attractive option. Milk ingredients can contribute to enhanced human resistance to infectious disease. By strengthening the gut barrier, milk constituents may prevent translocation. In addition, the immune response towards an infection is important in clearing pathogens. Milk constituents have also been shown to modify immune responses. Finally, the microbiota composition of the GI tract can influence the course of infections, by production of antimicrobial agents in the large intestine, competition on nutrients for bacterial growth and mucosal adhesion sites, and by modifying the immune response. Several milk constituents have been shown to modify the gut microbiota composition. All together, these activities can result in diminished pathogenic load and diarrhea. Until now, studies into activities of individual milk components are mainly limited to in vitro studies and animal infection models. Human evidence is scarce, more difficult to obtain, but highly needed. Because of evolutionary reasons, it can be expected that milk constituents acts synergistically in protecting against gastrointestinal infections.
A broad range of other antimicrobial agents are present in the bovine milk fat globule membrane. Pathogen decoy activity is the predominantly mentioned bioactivity. Constituents of the bovine milk fat globular membrane appear to be good inhibitors of rotavirus adhesion in vitro, but in-vivo evidence is scarce, except for MUC1 and lactadherin 4. Since norovirus and several bacterial pathogens can bind sialic acid 5 and sugar moieties 6, bovine milk fat globule constituents containing sialic acid and sugar groups, such as milk gangliosides, mucin and other glycosylated proteins, theoretically may act as

decoy, thus preventing adherence of these pathogens to the intestinal epithelium. However, this needs to be confirmed. Except for sphingolipids, the effect of the milk fat membrane constituents is less well studied for bacterial pathogens than for viral pathogens.
Sweet buttermilk powder, which is rich in milk fat globule membrane components, protected rats against Listeria-infection when compared to skim milk 7. This effect can be explained by the bactericidal effect of digestion products of sphingolipids, i.e. lysosphingolipids and sphingosine, or by the presence of milk fat globule membrane constituents that act as pathogen decoy, such as gangliosides, MUC1 and lactadherin.
So far, evidence from the milk fat globule membrane is mainly obtained from in-vitro data, whereas data from animal models is limited and scattered over different pathogens. Human evidence is scarce.

Study objective

We want to determine whether Lacprodan® PL-20 can effectively protect against enterotoxigenic E.coli induced travellers* diarrhea in humans. In the present double-blind, placebo-controlled, randomized parallel study, the effect of oral milk protein concentrate vs placebo will be studied on the resistance of humans to enterotoxigenic Escherichia coli infection (ETEC). Lacprodan® PL-20 is a milk protein concentrate rich in phospholipids and a source of phosphatidyl serine and sphingomyelin. The main hypothesis is that Lacprodan® PL-20 will improve human resistance to ETEC as measured by decreased fecal excretion of ETEC with time and less ETEC-induced daily fecal output.

Study design

Study design
Subjects, recruited from the Wageningen/Ede area, will participate in a randomized, double-blind, placebo-controlled, parallel intervention study of 4 weeks after receipt of signed informed consent. Subjects consume either Lacprodan® PL-20 or placebo. Subjects will be instructed to maintain their habitual diet and usual pattern of physical activity.

Dietary restrictions and replacing soy products
Subjects will be instructed to maintain their habitual diet, except for their dairy intake and intake of products with high amounts of prebiotic fibers and probiotics. Dairy has a high calcium content and contributes significantly to total daily calcium intake. Calcium can significantly reduce the gastro-intestinal symptoms induced by the ETEC strain1. To standardize and decrease dietary calcium intake of the subjects, low-calcium soy products will be provided to the subjects for the entire study. The low-calcium soy products will be purchased from Provamel Nederland BV. The soy products will be provided as single portions and can be stored at room temperature. Subjects will be instructed to consume the low calcium soy products at breakfast and dinner. The

subjects are not allowed to consume other dairy products during the study.

Lacprodan® PL-20
Lacprodan® PL-20 is a milk protein concentrate rich in phospholipids and a source of phosphatidyl serine and sphingomyelin. The placebo Miprodan® 30 is a sodium caseinate and is a powder of identical appearance. The placebo contains an identical amount of calcium but does not contain the bioactive components. Subjects are requested to mix the Lacprodan® PL-20 or the Miprodan® 30 powder twice daily in their soy drinks, once at breakfast and once at dinner, and during the entire study. Lacprodan® PL-20 will be supplied as a powder in sachets. The sachets have to be stored under cool and dry conditions to prevent deterioration due to humidity and high temperatures. The shelf life is a minimum of 12 months if kept under the prescribed storage conditions.

Oral ETEC Vaccine
After an adaptation period of 2 weeks to the intervention products, subjects will be infected with a single oral dose of attenuated ETEC strain E1392-75-2A at a dose of 1010 CFU (Bovee-Oudenhoven et al., 2003). Oral infection will occur between 11.00 h and 12.00 h AM. Before taking ETEC, subjects are not allowed to eat for 4 hrs and not to drink for 2 hrs. Thereafter, and under supervision of the project team, they will get a $NaHCO_3$ solution (100 ml 2% $NaHCO_3$) to neutralize the gastric acid. After 5 minutes, they get a fruit juice (100 ml) containing the ETEC strain at the above-mentioned dose. Subjects go home, but are not allowed to drink and eat for 1 hour.

Diaries and biological samples
Before and after infection, the subjects are asked to fill in a 2x24 hrs nutrition diary and report and estimate amounts of all foods and drinks eaten (online dairy; LimeSurvey2012). Bowel habits (defecation frequency) and frequency and severity of gastrointestinal symptoms (flatulence, bloating, abdominal pains and cramps) are self-recorded daily in an online diary (LimeSurvey2012), using Visual Analogue Scales (VAS; range 0-5 from none to severe) wherever appropriate.
Blood samples (10 ml) will be taken by qualified staff of a local hospital on 1 time point before and on 2 time points (day 3 and 14) after ETEC infection. Before (on 2 separate days) and after ETEC infection (on 5 separate days), 24 hrs fecal samples will be collected. All materials and information needed for proper collection of the fecal samples (stool collection kit) will be supplied by NIZO food research and delivered to the subjects. Feces will be frozen immediately after defecation. Subjects will be asked to store feces in mini-freezers, supplied by NIZO food research. Every 2-3 days, the frozen feces will be transported to the lab, weighed, homogenized, and analyzed for ETEC by QPCR. Homogenized fecal sub-samples will be frozen and stored (at -20 oC) for later analyses. Diarrhea will be quantified by analyses of fecal wet and dry weight. Results will be compared with self-reported information on stool consistency (Bristol stool scale).
In addition, before and after infection (on 2 separate days) 2 mL saliva will

be collected.

Intervention

The MIRAGE study is a parallel, double-blind, placebo-controlled 4-weeks intervention with a milk and whey protein concentrate in healthy volunteers. In this study, the effect of an intervention with Lacprodan® PL-20 vs placebo (Miprodan® 30) on several infection markers in response to an ETEC challenge is investigated. The infection markers of interest, the primary and secondary study outcomes, are mentioned below. Participants will be randomly assigned to the milk and whey protein concentrate or placebo group (n=30 per group). Subjects will be instructed to maintain their usual pattern of physical activity and their habitual food intake, but to standardize their dietary calcium intake. After an adaptation period of 2 weeks, subjects will be orally infected with a live, but attenuated, ETEC vaccine (strain E1392-75-2A; collection NIZO food research; dose will be 1010 CFU). Before and after infection, an online diary will be kept to record all food and drinks consumption (2x2 days) to assess the habitual dietary intake. The diary will also be used for daily recording of bowel habits and frequency and severity of gastrointestinal complaints. The following biological samples will be collected: 4x10 ml venous blood, a single fecal bolus (for screening) and 7x24 hrs feces. Blood is sampled for immune response analyses and the fecal samples are collected to quantify several infection- and immune system markers and to verify dietary calcium intake. Saliva is sampled three times before and after infection to quantify immune system markers.

Study burden and risks

Benefits for subjects to participate in the MIRAGE study
There are no direct benefits for the subjects from participation to the MIRAGE study. The single oral administration of the ETEC vaccine strain to the subjects offers no protection against E. coli infections in the future. Previous studies with this vaccine strain have shown that single oral administration leads to a rise of specific serum antibody titers, but the quality and quantity of the effect is considered inadequate for significant protection against subsequent infections. Only after repeated vaccinations protection would be induced against a very specific (and thus small) group of bacterial pathogens. Although there are not direct benefits for the study subjects, a positive study outcome can offer advantages for population groups in the future. When Lacprodan® PL-20 does improve resistance to ETEC infection, it will be possible to decrease intestinal infection incidence by providing relatively simple dietary advices, e.g. to travelers to tropical countries.

Safety information on ETEC strain
ETEC strain E1392-75-2A (supplier: Acambis, Cambridge, UK) is a spontaneous mutant unable to produce toxins. The strain obtained is 100% pure. Because of

its streptomycin-resistance it can be discriminated from other E. coli species that are part of the endogenous microbiotia and excreted in feces. ETEC E1392-75-2A is sensitive to ciproxin, which is a commonly used antibiotic for treatment of E. coli infections in humans. Vaccination experiments with this ETEC strain in humans are published by e.g. Tacket et al. (1997)2. In their study, after oral administration of 1010 CFU, 15% of the vaccinated persons suffered from self-limited, mild diarrhea with spontaneous recovery after 1-3 days. The most recent human intervention study at NIZO food research with this strain performed in 2010 showed that 79% of infected volunteers suffered from a mild and transient diarrhea for 1-3 days when orally dosed with log1010 colony forming units. Besides this, 74% of the volunteers experienced a mild abdominal pain, 63% reported bloating and 26% reported fever. Other symptoms, e.g. nausea and vomiting, were not reported and complications are not expected.

Safety information on Lacprodan® PL-20
Lacprodan® PL-20 is a milk cream powder reduced in triglycerid fat. This product is an approved food ingredient under the term *fat-reduced cream powder*. The product is manufactured, packaged and labelled according to the relevant EU-regulations for food and food ingredients, and/or FAO/ WHO Codex Alimentarius, when relevant. This includes that the milk/milk constituents used as raw material origins from healthy cows. The milk used in the production is included in monitoring programmes for undesirable substances, as required by regulations or HACCP-based risk assessment. The production plants are approved by the competent authorities and included in the EU-register of approved food establishments.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Healthy male subjects, aged 18-55 yrs, living in the Ede/Wageningen neighbourhood - Informed consent -Availability of internet connection - Willingness to replace habitual dairy product intake with the supplied lowcalcium soy products - Willingness to abstain from products with high amounts of prebiotic fibers and from products with probiotics (except for the supplied one) starting 1 month prior to study start - Willingness to give up blood donation from 1 month before the start of the experiment and during the entire experimental period.

Exclusion criteria

Current or previous underlying disease of the GI tract - Allergy to milk products or lactose intolerance (selfreported)- Allergy to soy products (self-reported) - Use of antibiotics, norit, laxatives (up till 6 months prior to inclusion), cholestyramine, acid burn inhibitors or immune suppressive (up till 3 months prior to inclusion), and pre- and probiotics (up till 1 month prior to inclusion)- High titer serum antibodies against ETEC (10 ml blood sample collected at screening). - Vegetarians - Vegans - Heavy alcohol use (>4 consumptions/day or >20/week) -Drug use

Study design

Design

Study type:	Interventional
Intervention model:	Parallel

Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)
Control:	Placebo
Primary purpose:	Prevention

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	02-10-2012
Enrollment:	60
Type:	Actual

Ethics review

Approved WMO

Date:	19-11-2012
Application type:	First submission
Review commission:	METC Wageningen Universiteit (Wageningen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL41768.081.12