The effect of Lactobacillus acidophilus NCFM on E. coli gastroenteritis

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

Summary

ID

NL-OMON34373

Source
ToetsingOnline

Brief title
PRETEC

Condition

- Gastrointestinal infections
- Bacterial infectious disorders

Synonym
gastroenteritis, traveler's diarrhea

Research involving
Human

Sponsors and support

Primary sponsor: Danisco Finland
Source(s) of monetary or material Support: Danisco Finland

Intervention

Keyword: enterotoxigenic E. coli, infection, Lactobacillus acidophilus, oral vaccin

Outcome measures

Primary outcome

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

• Total daily faecal output as marker of diarrhea

Secondary outcome

• Bowel habits

• Frequency and severity of gastrointestinal symptoms

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

dry weight)

• Opportunistic pathogens in faeces

• Calprotectin

• Specific serum antibody response to CFA-II

• Total faecal sIgA

Study description

Background summary

Foodborne infections occur frequently. In European countries 10-25% of the population suffer from at least one foodborne infection per year. This number increases sharply in 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 immuno-suppressive drugs). Treatment of foodborne 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. Enhancement of

human resistance to foodborne infections is an attractive option, besides improved hygiene measures. Probiotics can contribute to enhanced human resistance to infectious disease by excretion of anti-microbial components of by competing with pathogens for intestinal nutrients and mucosal adhesion sites. Lactobacillus acidophilus NCFM* is a probiotic strain originally isolated from the faeces of a healthy adult. The strain has been marketed without any known side effects since the 1970*s. Its complete genome sequence indicates the strain does not carry any known antibiotic resistance genes.6 Human studies have shown that the strain survives gastrointestinal transit.7,8 Thanks to the existence of a strain specific primer pair, it can be fully traced.8 The species L. acidophilus has also been granted Qualified Presumption of Safety status by the European Food Safety Authority.9 Human intervention studies have shown that consumption of the strain affects the composition3 and activity4 of the faecal microbiota. The strain also affects the small intestinal microbiota.10,11 Consumption of the strain, together with an oral cholera vaccine has been shown to lead to a more rapid immune response.5 In combination with another strain (Bifidobacterium lactis Bi-07), the strain has been observed to reduce diarrhoea.12 The strain also improved general health and well being in children. Although the diarrhea incidence was low in the study and no difference could be observed, the respiratory infection rate was dramatically reduced.13 In addition to these human studies, animal studies have shown that administration of the strain protects immune-deficient mice from Candida albicans infection14 and modulates various functions of the immune system.15
In the present double-blind, placebo-controlled, randomized parallel study, the effect of oral Lactobacillus acidophilus NCFM* vs placebo will be studied on the resistance of humans to enterotoxigenic Escherichia coli infection (ETEC). The main hypothesis is that Lactobacillus acidophilus NCFM* will improve human resistance to ETEC as measured by decreased faecal excretion of ETEC with time and less ETEC-induced daily faecal output.

Study objective

We want to study whether consumption of Lactobacillus acidophilus NCFM* can improve the resistance of humans to intestinal ETEC infection. An important parameter of intestinal resistance is the so-called colonization resistance. The colonization resistance is inversely related to the faecal excretion of a pathogen with time. The hypothesis is that Lactobacillus acidophilus NCFM* will decrease fecal ETEC excretion by 1 log10 units. As a consequence, diarrhea severity, as measured by total daily faecal output, will likely be reduced as well.

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 Lactobacillus acidophilus NCFM* or placebo. Subjects will be instructed to maintain their usual pattern of physical activity and their habitual diet, except for their dairy intake and products containing high amounts of prebiotic fibers and probiotics. Dairy has a high calcium content and contributes significantly to total daily calcium intake. To standardize and decrease dietary calcium intake of the subjects, in order to increase the efficacy of the vaccine, low-calcium milk and low-calcium vanilla custard will be provided to the subjects for the entire study (also see chapter 9). The low-calcium dairy products will be produced under GMP conditions and supplied by NIZO food research. The subjects are not allowed to consume other dairy products during the study. The probiotic Lactobacillus acidophilus NCFM* will be supplied as a powder in capsules. The placebo is also a powder of identical appearance and consists of the carrier material (microcrystalline cellulose). Subjects will be instructed to open a capsule and mix its contents with a glass of supplied dairy (150 ml per portion) and drink it immediately after preparation. This will be done twice daily, preferably at breakfast and dinner, and during the entire study. Each probiotic capsule contains 10E9 CFU Lactobacillus acidophilus NCFM*. The capsules will be provided in a bottle and can be stored at room temperature.
After an adaptation period of 2 weeks to the intervention products, subjects will be infected with single oral dose of attenuated ETEC strain E1392-75-2A at a dose of 10E10 CFU. 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 NaHCO3 solution (100 ml 2% NaHCO3) to neutralize the gastric acid. After 5 minutes, they get a fruit juice (50 ml) containing the ETEC strain at the above-mentioned dose. This is followed by consumption of 150 ml low-calcium milk with either added Lactobacillus acidophilus NCFM* or placebo, depending on assignment. Subjects go home, but are not allowed to eat and drink for 1 hour.
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. Bowel habits (defaecation frequency) and frequency and severity of gastrointestinal symptoms (flatulence, bloating, abdominal pains and cramps) are self-recorded daily in a diary, 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 2 time points after ETEC infection. Before (on 2 separate days) and after ETEC infection (on 5 separate days), 24 hrs faecal samples will be collected. All materials and information needed for proper collection of the faecal samples (stool collection kit) will be supplied by NIZO food research and delivered to the subjects. Feces will be refrigerated immediately after defecation, transported to the lab, weighed, homogenized, and analyzed for ETEC within 24 hours after defecation. Homogenized faecal 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).

Intervention

The PRETEC study is a dietary intervention with oral probiotics. Twice daily, preferably at breakfast and dinner, subjects consume 10E9 colony-forming units of Lactobacillus acidophilus NCFM. The probiotic is a dry powder and has to be mixed with the supplied dairy products (low-calcium milk and vanilla custard, 150 ml each). The control group receives a powder of identical appearance which consists of carrier material only, to be used with the supplied dairy products as mentioned above.

Study burden and risks

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 faeces. 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 (Vaccines against enterotoxigenic E. coli infections. In: New generation vaccines. Eds. Levine et al., Marcel Dekker Inc., New York, 1997; 875-883). 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. In a previous ETEC study of NIZO food research (Bovee-Oudenhoven et al. Gastroenterol 2003; 125: 469-476) using the same infection dose, almost all subjects consuming dietary placebo experienced mild diarrhea for 2 days. Reported infection symptoms were: stomach pain (34%), headache (25%), bloating (22%) and a slight rise of body temperature (13%), indicating a mild type of foodborne infection. Other symptoms, e.g. nausea and vomiting, were not reported and complications are not expected.

Safety information on Lactobacillus acidophilus NCFM*:
The strain has been marketed without any known side effects since the 1970*s. Its complete genome has been sequenced and published (Alterman E et al. Proc Natl Acad Sci USA 2005; 102: 3906-3912). The sequence indicates that the strain does not carry any known antibiotic resistance genes. The species L. acidophilus has also been granted Qualified Presumption of Safety status by the European Food Safety Authority (Andreoletti O et al. EFSA J 2008; 923: 1-48). Thanks to the existence of a strain specific primer pair, it can be fully traced (Ouwehand AC et al. Br J Nutr 2009; 101: 367-375).

Benefits for subjects:
There are no direct benefits for the subjects from participation to the PRETEC

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 Lactobacillus acidophilus NCFM* does improve resistance to ETEC infection, it will be possible to decrease intestinal infection incidence by providing relatively simple dietary advices and probiotic products, e.g. to travelers to tropical countries.

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 20-55 yrs, living in the Ede/Wageningen neighbourhood
- Informed Consent

Exclusion criteria

- Measurable serum antibody titer against enterotoxigenic E. coli
- Carrier of streptomycin-resistant E. coli (in faeces)
- Presence or former gastrointestinal diseases
- Lactose intolerance
- Use of antibiotics, norit, laxatives (up till 6 months prior to inclusion), cholestyramine, acid burn inhibitors or immune suppressors (up till 3 months prior to inclusion), and pre- and probiotics (up till 1 month prior to inclusion)
- Vegetarians
- Heavy alcohol use
- 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):	04-11-2010
Enrollment:	42
Type:	Actual

Ethics review

Approved WMO Date:	02-11-2010
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	NL32716.081.10