A randomized, placebo-controlled, double blind human trial into the effect of Protecflor on gastroenteritis caused by attenuated E. Coli

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

Summary

ID

NL-OMON37365

Source
ToetsingOnline

Brief title
PROTETEC

Condition

- Gastrointestinal infections
- Bacterial infectious disorders

Synonym
gastroenteritis, traveler's diarrhea

Research involving
Human

Sponsors and support

Primary sponsor: Lallemand Human Nutrition
Source(s) of monetary or material Support: Lallemand Institute Rosell

Intervention

Keyword: enterotoxigenic E. coli, infection, oral vaccin, probiotics

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)

- Specific serum antibody response to CFA-II

Tertiary study outcomes (performance will depend on results primary and secundary markers)

- Opportunistic pathogens in faeces

- Calprotectin

- Total faecal sIgA

Study description

Background summary

The incidence of gastrointestinal infections is very high. In European countries 10-25% of the population suffers from at least one foodborne infection per year. Recent studies suggest that this incidence is underestimated, and that even 90% of the population may suffer from infectious diarrhea each year. Usually these infections are self-limiting and only some days of diarrhea, stomach pain, nausea and vomiting occur. However, such ordinary infections can be life-theatening 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.

Protecflor is a blend of probiotic bacteria (Lactobacillus helveticus Rosell-52, Lactobacillus rhamnosus Rosell-11, Bifidobacterium longum Rosell-175 and probiotic yeast (Saccharomyces boulardii). Each individual strain has been consumed in nutritional and dietary supplements for more than 20 years without any reported side effect. Its complete genome sequence indicates the strain does not carry any known antibiotic resistance genes. Using an in-vitro digestion system (IViDiS), all strains in Protecflor® have been shown to survive gastrointestinal conditions (Tompkins et al,. 2011). Survival was best when Protecflor was given 30 minutes before a meal or together with a meal. (Tompkins et al., 2011). Thanks to the existence of strain specific primer pairs, it can be fully traced. All microorganisms in Protecflor have also been granted Qualified Presumption of Safety status by the European Food Safety Authority .

S. boulardii has been shown to bind pathogenic bacteria, such as E. coli in vitro (Gedek , 1998), forming agglutinates that may easy be eliminated through the gut. S. boulardii reduced the duration of rotaviral diarrhea in children (Grandy et al., 2010). In addition, human intervention studies with children have shown that S. boulardii reduced 1) the duration of non-specified diarrhea (Kurugol et al., 2005, Htwe et al., 2008), 2) the number of children with prolonged diarrhea (Villarruel et al, 2007) and 3) the number of days of hospitalization (Kurugol et al, 2005). In addition, S. boulardii reduced the incidence of diarrhea in travelers (Kollaritsch et al, 1993).

The lactic acid bacteria L. helveticus Rosell-52 and L. rhamnoses Rosell-11 has been shown to bind to host epithelial cells and to reduce the binding of E. coli to these epithelial cells (Sherman et al., 2005). The mixture of these probiotics has been shown to reduce the duration of bacterial and/or viral diarrhea in children (Tlaskal 1995, Tlaskal 2005)) and diminished the incidence of Citrobacter freindi and Pseudomonas aeruginosa (Tlaskal, 1995))

The efficacy of Protecflor® in protecting against ETEC diarrhea in rats was compared with that of solely S. boulardii and with a mixture of the lactic acid bacteria L. helveticus Rosell-52, L. rhamnoses Rosell-11, and B. longum Rosell-175. Whereas administration of solely S. boulardiii and the lactic acid bacteria mixture had minor effects on E. coli-induced diarrhea, Protecflor® improved stool consistency and shortened the duration of diarrhea (Bisson 2009). The protection against diarrhea was accompanied with a decreased secretion of the pro-inflammatory cytokines IL1-α, IL-1β, IL-6, IFN-γ and TNF-α, and an enhanced secretion of anti-inflammatory cytokines IL-4 and IL-10 (Bisson 2009). Protecflor® also showed synergistic effects in preventing weight loss in E. coli-infected rats (Bisson 2009). Protecflor tended to shorten duration of rotaviral diarrhea in Bolivian children (Grandy et al., 2010).

In the present double-blind, placebo-controlled, randomized parallel study, the effect of oral Protecflor vs placebo will be studied on the reistace of humans to enterotoxigenic Escherichiea coli (ETEC) infection. The main hypothesis is that Protecflor 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 Protecflor 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 Protecflor will decrease fecal ETEC excretion by 1 log10 units. As a consequence, diarrhea severity, as measured by total daily fecal 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 Protecflor® or placebo. Subjects will be instructed to maintain their usual pattern of physical activity and 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. To standardize and decrease dietary calcium intake of the subjects, in order to increase the efficacy of the vaccine2, low-calcium soy products will be provided to the subjects for the entire study (also see chapter 9). The low-calcium soy products will be purchased from Bio Soya Desert Provamel Alpro Soja Nederland BV. The subjects are not allowed to consume other dairy products during the study. The probiotic Protecflor® will be supplied as a powder in capsules. The placebo is also a capsule made of the same material (hydroxypropyl-methylcellulose and titanium oxide) and contains a

powder of identical appearance and consists of the carrier material (potato starch). Subjects will be instructed to consume the low calcium soy products at breakfast and dinner. In addition, subjects are requested to ingest a capsule twice daily, one at breakfast and one at dinner, and during the entire study. Each probiotic capsule contains 5 x109 Protectflor®. 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 a single oral dose of attenuated ETEC strain E1392-75-2A at a dose of 1010 CFU.2 Oral infection will occur between 10.00 h and 11.00 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 NaHCO3 solution (100 ml 2% NaHCO3) 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.

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 1 time point before 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 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. Subjects will be asked to store feces in mini-freezers, supplied by NIZO food research. Every 3 days, the frozen feces will be transported to the lab, weighed, homogenized, and analyzed for ETEC by QPCR 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). A time schedule of the PROTETEC study is added as appendix A.

Intervention

The PROTETEC study is a dietary intervention with oral probiotics. Twice daily, at breakfast and dinner, subjects consume 5 x 10E9 colony forming units Protecflor. The probiotics are supplied as capsule containing dry powder, and need to be consumed together with the supplied soy products. The control group receives a placebo capsule of identical appearance which consists of carrier material only, to be used with the supplied soy 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. 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 10E10 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 Protecflor:
The active substance in Protecflor consists of the probiotic yeast Saccharomyces cervisiae var boulardii (CNCM I-1079) combined with 3 strains of lactic acid bacteria Lactobacillus helveticus R0052 (CNCM I-1722), Lactobacillus rhamnosus R0011 (CNCM I-1720) and Bifidobacterium longum R0175 (CNCM I-3470). Each of the individual strains in Protecflor have been consumed in nutritional and dietary supplements for more than 20 years without any reported side-effects and have not been identified in infections. All strains are registered in the National Collection of Microorganism Cultures (CNCM) at the Intitute Pasteur in France. All four species present in Protecflor haven been granted Qualified Presumption of Safety status by the European Food Safety Authority (EFSA 2011). The International Dairy Federation in collaboration with the European Food and Feed Cultures Association (IDF/EFFCA 2002) assembled a list of microorganisms with a documented history of safe use in food. Saccharomyces cervisiae var boulardii, Lactobacillus helveticus, Lactobacillus rhamnosus and Bifidobacterium longum are on that list. The genomes of the 4 microorganisms in Protecflor have been sequenced and annotated (internal results Lallemand). The genome for Lactobacillus rhamnosus has been published (Tompkins et al, 2012). The four strains in Protecflor have been tested for antibiotic resistance genes using minimal inhibitory concentration breakpoints, according to the recommendations of the FEEDAP Panel (2008). None of the strains carried any known antibiotic resistance gene (internal results Lallemand). Further testing confirmed the lack of antibiotic resistance genes (internal results Lallemand). Primer sets exists for all four microorganisms in Protecflor, which allow the detection and enumerations of the strains in

various samples (internal results Lallemand).

Benefits for subjects:
There are no direct benefits for the subjects from participation to the PROTETEC 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 Protecflor® 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
- Availability of internet connection
- Willingness to replace habitual dairy product intake with the supplied low-calcium 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 (self-reported), since the capsules may contain milk traces from culture media
- 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:	24-08-2012
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	NL40301.081.12