STUDY OF THE USE OF PROBIOTIC FOODS AS A COMPLEMENT OF THE CONVENTIONAL ANTIBIOTIC-THERAPY FOR THE TREATMENT OF HELICOBACTER PYLORI INFECTION IN CHILDREN AND IT'S USE AS A PROPHYLACTIC THERAPY IN THE REINFECTION BY THIS PATHOGEN

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XA0056163

Abstract

Helicobacter pylori infection is very frequent in children in developing countries. Studies on eradication regimens and its complications are not well documented. The European Helicobacter pylori Study Group strongly recommends that treatment should be with proton pump inhibitor based triple therapy, consisting of a proton pump inhibitor and two of the following: clarithromycin, a nitroimidazole (metronidazole or tinidazole) and amoxycillin in various combinations. Recent advances in probiotic research show much promise in a new product development of functional foods based on milk. Among the reported beneficial effects of consuming certain strains of cultures or their metabolites, or both are control of ulcers related to Helicobacter pylori. Kefir seems to be a potential probiotic to control Helicobacter pylori infection. In this study 2 groups of 10 children each which demonstrate to be Helicobacter pylori positive will be treated as follows: Group 1: antibiotic treatment + placebo (fluid milk) and Group 2: antibiotic treatment + kefir. The Triple Therapy consists in the combination of two antibiotics (amoxycillin and clarithromycin) with a proton pump inhibitor (Lansoprazole). In all the cases, the post-treatment control will be performed by the ^{13}C UBT 2 months after the end of the treatment. Once the infection is eradicated, the group who received the antibiotic-therapy with milk (placebo) as well as the group who received the antibiotic-therapy with the probiotic under study will continue with the administration of the milk and/or probiotic food during one year. During this period, the children will be submitted to post-treatment controls performed by the ^{13}C UBT every three months. We expect to find that the group that received the triple therapy in combination with the probiotic food (kefir) would have less recidiva rates for the Helicobacter pylori infection than the group that received the triple therapy with the placebo (fluid milk).

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Helicobacter pylori infection is one of the most common gastrointestinal bacterial diseases worldwide with a 30 to 50% incidence in the population. In developed countries, H. pylori infection is not frequent in children and rises as a function of age, reaching a 30% of incidence in people of more than 60 years old and remains constant in older people.

In developing countries, the incidence is approximately 70%, showing that most of the population is infected, independently of age [1-5].

Even though, Helicobacter pylori infection is very frequent in children in developing countries, studies on eradication regimens and its complications are not well documented. For example, triple therapy with two antibiotics and an antisecretory drug has been seldom tried in children because the compliance is often poor so that the eradication rate is often similar to that produced by dual therapy [6].

The factors that lead to treatment failure are [7]:

1.1 Patient related factors

- **Poor compliance**: Patient compliance plays an important role in the success of H. pylori eradication treatment.
- **Bacterial resistance**: Bacterial resistance to metronidazole or clarithromycin is an important factor leading to treatment failure.

- **H. pylori strains**: The cagA- status of bacterial strains is also a risk factor for treatment failure. In a multicentre study it was found an eradication of 87% for the cagA+ strains and a 69% in the cagA- strains.

1.2 Treatment related factors

- ***Components of a regimen***: The components of a treatment regimen also play an important role in H. pylori eradication, such as which drugs are selected, the number and doses of medications used in the combination, dosing frequency, and treatment duration.

Eradication of Helicobacter pylori infection in children may be useful both to induce symptom remission and to prevent later complications in adulthood [6].

Studies on symptoms associated with H. pylori infection in children are scarce, but some report the presence of alarm symptoms, such as malabsorption with weight loss, delay in weight gain, short stature, iron deficiency anemia, or recurrent diarrhea and malnutrition in infected children [6].

The European Helicobacter pylori Study Group (EHPSG) strongly recommends that treatment should be with proton pump inhibitor based triple therapy, consisting of a proton pump inhibitor and two of the following: clarithromycin, a nitroimidazole (metronidazole or tinidazole) and amoxycillin in various combinations [8].

Even though guidelines on the management of H. pylori infection in adults have been performed [8-9], guidelines on the management of H. pylori infection children are still lacking and there may be some advantages to curing the infection in childhood. Although there are not enough data on children of H. pylori infection, we should bear in mind that we still do not know the whole clinical picture of this chronic infection, and the longer the duration of the disease the worse seems to be the prognosis [6].

On the other hand, the increase of the treatment of Helicobacter pylori result in an increase in complications such as antibiotic-associated diarrhea. It has been reported a case of a patient who developed Clostridium difficile colitis after treatment for Helicobacter pylori infection with metronidazole, amoxycillin, H2 blockers and bismuth subsalicylate [10]. Antibiotic therapy is often unsuccessful and most often associated with risks of significant adverse effects, being the consequence of intestinal microflora disorders [11].

Recent advances in probiotic research show much promise in a new product development of functional foods based on milk. Among the reported beneficial effects of consuming certain strains of cultures or their metabolites, or both are enhanced immune response, balancing of colonic microbiota, vaccine adjuvant effect, reduction of fecal enzymes implicated in cancer initiation, treatment of diarrhea associated with travel, antibiotic therapy, control of rotavirus and Clostridium difficile, control of ulcers related to Helicobacter pylori, reduction of serum cholesterol, antagonist against food-borne pathogens and tooth decay organisms, and amelioration of lactose malabsorption symptoms. The mode of action in most cases seems to involve modulation of ecosystem of the gastrointestinal tract of the host. Several strains belonging to genera Enterococcus, Lactobacillus, and Bifidobacterium that have desirable clinical benefits are now available. They are being incorporated in yogurts, dairy snacks, breakfast foods, drinks, refrigerated desserts, cheeses, spreads, frozen desserts, and unfermented sweet cultured milk [12].

In cases of Helicobacter pylori associated pathology, the deficiency of Lactobacillus sp. in the stomach was established. It was also observed a decrease in the population level of Bifidobacterium sp. with the simultaneously increase of opportunistic enterobacteria and changes in the local immunity. So, it can be concluded that the correction of microecological and immune disturbances with probiotic preparations containing bifidobacteria and lactobacilli, yielded good results [13]. On the other hand, it has been demonstrated that a certain amount of time is necessary for Helicobacter pylori to contact with the gastric epithelium and that the composition of the flora is important for the establishment of Helicobacter pylori infection [14].

On the basis of clinical criteria, the use of probiotics containing lacto and bifidobacteria, simultaneously with "triple" antibacterial therapy (antibiotics, metronidazole and bismuth salts) has been found to produce curative effect in the treatment of Helicobacter pylori associated gastroduodenal pathology in children. The prescription of bifidobacteria containing probiotics is recommended at early stages from the beginning of etiotropic therapy [15].

Kefir is the product of the fermentation of milk with kefir grains. The composition of kefir grains is variable and not well defined. It is described as a symbiotic association between lactic and acetic bacterias and yeast.

Fresh kefir demonstrates to have a stimulatory effect on the motor and emptying function of the gastric stump. So, the dietetic management of the patients with Helicobacter pylori colonization should improve the treatment [16].

So, nutriceuticals and probiotics have demonstrated interesting in vitro activity against Helicobacter pylori. Children rarely have symptoms of infection and, therefore, are a suitable group in which to assess different nonaggresive therapies [17].

2. MATERIALS AND METHODS

2.1. Protocol design

Study on children (1-12 years old)

The study of the ^{13}C UBT [18] will be performed in children, age ranging between 1 and 12 years old, who assist to the gastroenterology visit and that refer symptoms in agree with the Helicobacter pylori infection. It will be selected 20 children who demonstrate to be Helicobacter pylori positives according to the studied methodology. The parents or tutors of the child who will be part of the study must sign the information and informed written consent protocols according to the Helsinki declaration. The patients will be separated into 2 groups of 10 children each. Each group will receive:

Group 1: *10 children receiving antibiotic treatment + placebo (fluid milk)*
Group 2: 10 children receiving antibiotic treatment + kefir

The Triple Therapy consists in the combination of two antibiotics (amoxycillin and clarithromycin) with a proton pump inhibitor (Lanzoprazol).

In all the cases, the post-treatment control will be performed by the ^{13}C UBT 2 months after the end of the treatment. Once the infection is eradicated, the group who received the antibiotic-therapy with milk (placebo) as well as the group who received the antibiotic-therapy with the probiotic under study will continue with the administration of the milk and/or probiotic food during one year. During this period, the children will be submitted to post-treatment controls performed by the ^{13}C UBT every three months. The study will be double blinded and the results evaluated by independent researchers. In this way it will be possible to evaluate the rate of re-infection for both groups.

2.2. ^{13}C-Urea Breath Test

To perform the ^{13}C-UBT, the children must be fasting for at least 6 hours. Two samples of exhaled air will be taken previous to the ingestion of the labeled solution to determine the basal counts. Then, 25 ml of water containing 65 mg of ^{13}C-urea will be administered. Breath samples are taken at 30 and 45 minutes after the ingestion of the labeled solution in hermetically sealed exetainers.

2.3. Measurements

Each sample of exhaled air will be measured in a mass spectrometer coupled to a gas chromatographer (GSMS) and the results will be expressed as a $^{12}C/^{13}C$ ratio.

3. RESULTS

Up to the moment, the personnel involved in the study are being trained and the production and quality controls of the probiotic under study are being carried out.

On the other hand, the study protocol has begun with the evaluation of the children selection criteria, children exclusion criteria, dose and diagnosis protocol. The information and consent protocols for the parents or tutors of the children that are going to be in the study are being written.

Selection and treatment of the children will take at least 1.5 years. We expect to find that the group that received the triple therapy in combination with the probiotic food (kefir) would have less

recidiva rates for the Helicobacter pylori infection than the group that received the triple therapy with the placebo (fluid milk).

4. PLANS FOR FUTURE WORK

The project is to be carried out within three years. The general schedule is:

- **First year**: Training and capacitating of the personnel involved in the project.
  Production and quality control of the probiotic foods
  Initiation of the protocol of the study:
  a) Children selection criteria
  b) Children exclusion criteria
  c) Information and consent protocols for the parents or tutor of the children and the referring physician
  d) Dose protocol
  e) Diagnosis dose protocol
  f) Selection of the antibiotic-therapy

- **Second year**: Execution of the protocol of the project (see protocol design).

- **Third year**: Analysis and Processing of the experimental results. Discussion and conclusions.

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