TREATMENT OF CHILDREN WITH H. PYLORI INFECTION WITH PROBIOTICS: COMPARISON WITH CONVENTIONAL METHODS.

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Summary

Helicobacter pylori colonizes the gastric mucosa of a high proportion of the population of the less developed countries since an early age. In the developed countries this occurs at a later age and with less frequency. This pathogen causes mostly asymptomatic infection but in a proportion of the population it is associated with chronic gastritis, peptic ulcer, atrophic gastritis. A subset of these individuals will eventually develop gastric carcinoma. For this reason there has always been considerable interest in developing innocuous, fast, inexpensive, sensitive, specific and noninvasive methods for diagnosis. The ^{13}C-urea breath test (^{13}C-UBT) satisfies most of these requirements. Eradication of H. pylori is accomplished by administration of proton pump blockers and a variety of antibiotics singly or in associations. The rate of success is rather high but treatment is long, expensive, it has secondary effects and it increases bacterial resistance. For this reason it is worth looking at other forms of treatment. Probiotics have demonstrated capacity to stimulate defensive mechanisms and to inhibit and even kill H. pylori in vitro and in animal experiments. We propose to conduct a study in which children 10 to 18 years of age colonized by H. pylori will receive a probiotic (Lactobacillus GG) twice daily for 4 or 8 weeks and the rate of infection will be assessed by the ^{13}C-UBT. Local immune responses will be evaluated by measuring quantitatively total salivary sIgA and specific sIgA. A group of children who will not receive Lactobacillus GG will serve as controls for all procedures. We believe that Lactobacillus GG will eradicate H. pylori and thus make it possible to treat this infection without recourse to antibiotics.

Helicobacter pylori must be the commonest pathogen in the world since in some of the most populous areas of the world almost 90 per cent of the population may harbour it in their stomachs by the time they reach early adulthood [1,2]. The prevalence of the infection in Chile has been determined to be about 70 per cent by Vial and coworkers [3]. Studies in Peru revealed even higher prevalence numbers at comparable ages [4]. On the basis of epidemiological evidence, acquisition of HP colonization in Chile is associated with consumption of untreated drinking water, raw vegetables and shellfish.

Because of this early occurrence of infection, the probabilities of the Chilean population for developing gastric malignancies should be very high; this is supported by data that show that, indeed, Chile does have one of the highest rates of gastric malignancies in the world [5]. At the same time, the application of endoscopic techniques to pediatric age subjects has revealed that the incidence of gastro-duodenal pathologies is rather high, with many individuals having lymph follicle hyperplasia, various forms of gastritis and peptic disease (gastric and duodenal ulcers). Early infection without subsequent eradication is probably one of the (main?) risk factors for gastric cancer [6].

During the last two decades Chile has undergone a very rapid epidemiological transition characterized by a steep decline of infant mortality (from 71 per thousand in 1971 to 9.4 in 1999); the decline of infantile malnutrition which decreased from 68 per cent of the population under one year of age in 1968 to less that 4 per cent in 1998 with a majority of mild cases, and of the incidence of preventable infectious diseases to levels comparable to those of developed countries. At the same time, chronic non-transmissible pathologies have increased proportionally as the lifespan of the population has reached 72 years for males and 76 for females. About 95 per cent of the population has now access to drinking water of acceptable quality and close 92 per cent of the households are connected to a sewerage system. Under these circumstances it becomes interesting to know what effect this may have on the prevalence of H. pylori infection as a powerful cohort effect may be detected by comparing the prevalence of H. pylori infection in individuals of the older and the younger generation [7].

In the less developed countries, the high levels of microbiological contamination of the environment are associated with alterations of intestinal morphology and deterioration of absorptive function; this condition has been called chronic environmental enteropathy and its intensity may be as great as that observed in Haitians [8] or as mild as that observed in Chileans [9]. As environmental conditions improve, albeit partially as it has been happening in Chile, disturbances of small intestinal function may become so mild that detection by means of the tests applied in the study of this alteration

are not sensitive enough. It may be postulated that study of the prevalence and incidence of H. pylori infection may represent a useful tool to monitor the effectiveness of environmental sanitation.

Eradication of H. pylori is accomplished by combining a proton pump inhibitor, one or two antibiotics and/or, in some schemes, a bismuth salt. Compliance is an important concern and the treatment should attain an equilibrium between duration and compliance, taking into account that in general it is difficult to subject children to prolonged treatments [10,11]. Antibiotic treatments have the inconvenience of generating resistant strains and therefore, it seems useful to look for other methods of treatment that do not involve antibiotic use and, therefore, do not involve the risk of transmitting some form of resistance to the treatment.

Probiotics have been considered as a possible tool for this purpose. The reasons are multiple. Probiotics have been shown in some studies to enhance immune responses to some antigens: L. Johnsoni LA1 increased the serum IgA and of salival sIgA responses of adults to immunization with S. typhi Ty21A [2]. The same probiotic increased the phagocytic activity of neutrophils. Other strains increased the numbers of circulating B lymphocytes [13]. There is also ample evidence that some strains of lactobacilli shorten the duration of episodes of acute diarrhea. Lactobacillus GG shortened the diarrheal phase of rotavirus infection in a number of studies in a variety of settings; the shortening was of about one day and the severity of the disease was lessened [14]. The same effect is shared by other probiotic species and strains [15]. However, Enterococcus SF68 did not exhibit any effects on watery diarrrhea caused by Vibrio cholera or by toxigenic Escherichia coli [16]. By comparison, E. faecium did shorten the duration of diarrhea in a placebo-controlled study in adults [17]. Some probiotic lactobacilli seem to shorten the duration of antibiotic-associated diarrhea caused by Clostridium difficile although it is possible that the small number of patients involved in these studies may have contributed to obscure the real effects. A positive effect has been demonstrated for Saccharomyces boulardii, a unicellular yeast [18]. On the other hand, clear protective effect has been shown in the prevention of traveler's diarrhea [19]. In view of these antecedents it is logical to propose the use of probiotics for prevention or treatment of H. pylori infection. A review of the literature shows that there are few publications in this respect.

The effect of probiotics on H. pylori has been evaluated in a few studies. Some of these are in vitro evaluations: Bhatia and coworkers demonstrated that L. acidophilus inhibited strains of Campylobacter (Helicobacter) pylori isolated from patients with acid-peptic disease. Lactic acid at concentrations of 1 per cent or 3 per cent inhibited H. pylori growth but this was not achieved by 0.5 or 1 per cent hydrogen peroxide. The sonic extract of the Lactobacillus had no effect [20]. Midolo and coworkers [21] observed that lactic, acetic and hydrochloric acids inhibited the growth of H. pylori in a concentration-dependent manner, lactic acid being the most active. Of the probiotic strains assayed in vitro, 6 strains of L. acidophilus and one strain of L. casei subsp rhamnosus also inhibited H. pylori growth while other probiotics such as B. bifidus, Pediococcus pentosaceus and L. bulgaricus did not. The authors postulated that the capacity of the bacteria to produce lactic acid was the main factor in the inhibition of the growth of H. pylori.

Studies carried out in laboratory animals [22] showed that administration of L. salivarius to gnotobiotic mice prevented gastric colonization by H. pylori and could even eliminate monocontamination by H. pylori in previously gnotobiotic mice; the release of IL-8 in vitro, that was used as a marker of inflammation was also decreased by L. salivarius. A similar effect of inhibiting the growth of H. pylori was demonstrated in vitro by Aiba and co-workers [23]. It has been shown both in vitro and in vivo in mice that the spent culture supernatant of Lactobacillus acidophilus strain LB decreases the viability of H. pylori in vitro in a way that is independent of pH and concentrations of lactic acid as well as the adhesion of the bacteria to a gastric epithelium cell line. In experiments in mice the spent culture supernatant inhibited stomach colonization by Helicobacter felis and the appearance of histological lesions in the gastric mucosa. The same preparation inhibited the urease activity of the surviving live bacteria [24]. It has been recently shown that the supernatant of cultures of Lactobacillus johnsonii strain LA1 interferes with the in vivo growth of H. pylori irrespective of its binding to epithelial cells. In infected volunteers who received 20 mg of omeprazole four times every day and who received either the supernatant or a placebo as a whey-based drink or a placebo, the LA1 supernatant induced a significant decrease ($p<0.03$) in urea breath test values and this persisted for up to six weeks after treatment [25]. This suggests [24,25] that probiotics release into the culture medium molecule(s) that exert an inhibitory effect on H. pylori. However, the persistence of this effect for some time after the probiotics has been ingested, in this case LA1, may be indicative of an immunological effect, as suggested by some of the evidence discussed elsewhere [12,13].

We propose to carry out a study to investigate the effects of the ingestion of Lactobacillus GG on the gastric colonization by H. pylori in children. The working hypothesis is that in children colonized with H. pylori regular ingestion of a milk product containing Lactobacillus GG will decrease the gastric colonization by the pathogen.

The aim of the project will be to evaluate in school age children colonized by H. pylori by means of the ^{13}C-urea breath test (UBT) the effect of Lactobacillus GG the evolution of this infection.

The specific aims will be:

1). To determine the presence of H. pylori in school-age children 10 to 18 years of age by means of the UBT;

2). To determine the severity of the colonization after 4 and 8 weeks of ingesting Lactobacillus GG;

3). To determine the severity of the colonization 4 weeks after interrupting the ingestion of Lactobacillus GG;

4). To determine the effect of the ingestion of Lactobacillus GG on the local immune response by measuring total and H. pylori-specific sIgA levels in saliva.

Subjects and procedures

The subjects of this study will be children attending a school in the South Eastern area of Santiago. Parents or legal guardians will be informed about the aim and scope of the project, its methods and requirements. Those who agree to participate will sign a written consent form. Children will belong to either sex and will be H. pylori positive as demonstrated by the UBT. Children who suffer from gastroduodenal ulcer or who are undergoing treatments with antibiotics, antacids or prokinetic drugs will be excluded.

Experimental design

A total of 114 children positive for H. pylori by the UBT will be included in the study; a dropout rate of about 10% has been considered in the calculation of the sample size. The study considers two groups of 68 children each; the experimental group will receive twice daily (at the beginning and at the end of the school-day) and under supervision of a teacher 80 ml of a product that contains at least 10^7 Lactobacillus GG per ml. The control group will receive the same product but without Lactobacillus GG. A second UBT will be performed after 4 weeks to both groups. The experimental group will then be divided into two subgroups (34 children each). Group 1 will continue receiving the Lactobacillus GG-containing product for an additional 4 weeks while the other group (Group 2) will not receive it. At the end of this period a third UBT will be performed to all children (experimentals and controls). Contemporarily with the UBT samples of saliva will be collected to evaluate the secretory immune response.

A tolerance survey will be conducted at weekly intervals and any symptoms declared by the participants will be recorded. Twice the amounts of product to be consumed on weekends will be delivered to the parents of the students on Fridays; the additional product is intended to avoid "dilution" within the family group.

Sample sizes have been calculated with "Primer" software. If we want to detect a decrease of delta $^{13}C_{PDB}$ of 50% between sample 1 (admission) and sample 3 (week 8), with an α error of 5% and a β error of 80%, and considering a variability of 1 standard deviation, then the total number of children that have to be included in the protocol is 68. The number of children in the control group will be 34.

Techniques

The ^{13}C-urea breath test (^{13}C-UBT)

For this test all children will be fasting overnight and sitting comfortably for the duration of the test (about 35 minutes). A basal sample of expired air will be collected by duplicate in a tube (t_0). The subject will then ingest a glass of orange juice (to delay gastric emptying) and after 5 minutes will ingest 75 mg of ^{13}C-urea dissolved in 50 ml of water. After 30 minutes a second breath sample will be collected, also by duplicate, in another tube (t_{30}). Enrichment with ^{13}C of expired CO2 in relation to 12C will be determined by mass spectrometry. A delta $^{13}C_{PDB} > ^5$ will be considered as positive.
Before and after the UBT, an uncontaminated sample of unstimulated saliva will be collected with a plastic Pasteur pipette; the sample will be centrifuged for 10 minutes at 3500 rpm and it will be frozen at –80°C until processed. Total sIgA and sIgA specific for H. pylori will be measured by ELISA.
For each period the eradication rate, the average of the delta $^{13}C_{PDB}$ will be measured as a reflection of gastric colonization by H. pylori. The quality of the immune response will be assessed by measuring the secretory immunoglobulin levels in saliva.

Statistical analysis

The results of the delta $^{13}C_{PDB}$ > of each interval and of each group of individuals will be compared by analysis of variance for repeated samples with subsequent contrast analysis if necessary. Comparisons between the experimental and control groups will be by multivariate variance analysis.

Results expected

We expect that the regular intake of Lactobacillus GG will decrease the intensity of the gastric colonization by H. pylori and that this effect should persist even after consumption of the product has ended. We also expect to detect the stimulation of mucosal humoral immune responses.

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