Effect of regular ingestion of *Saccharomyces boulardii* plus inulin or *Lactobacillus acidophilus* LB in children colonized by *Helicobacter pylori*

MARTIN GOTTELAND[1], LAURA POLIAK[2], SYLVIA CRUCHET[1] & OSCAR BRUNSER[2]

[1]*Laboratory of Microbiology, and* [2]*Laboratory of Ultrastructure, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile*

Abstract

Aim: To evaluate the effect of a probiotic, *Lactobacillus acidophilus* LB (LB), or a synbiotic, *Saccharomyces boulardii* plus inulin (SbI), on *Helicobacter pylori* (Hp) colonization in children. *Subjects and methods:* A clinical trial was carried out in a school from a low socio-economic area of Santiago. Two hundred and fifty-four asymptomatic children (8.40 ± 1.62 y) were screened for Hp by the ^{13}C-Urea Breath Test (^{13}C-UBT). Hp-positive children were randomly distributed into three groups to receive either antibiotic treatment (lanzoprazole, clarythromycin and amoxicillin) for 8 d, or SbI or LB daily for 8 wk. A second ^{13}C-UBT was carried out at this time. Spontaneous clearance was evaluated in the same way in 81 infected, untreated children. The differences in the $\delta^{13}CO_2$ over baseline values before and after treatments (ΔDOB) were evaluated. *Results:* 182 subjects (71.7%) were colonized by Hp, and 141 of them completed their treatment (22.5% dropout). Hp was eradicated in 66%, 12% and 6.5% of the children from the Ab, SbI and LB groups, respectively, while no spontaneous clearance was observed in the children without treatment. A moderate but significant difference in ΔDOB was detected in children receiving living SbI (-6.31; 95% CI: -11.84 to -0.79), but not in those receiving LB ($+0.70$; 95% CI: -5.84 to $+7.24$).

Conclusion: *S. boulardii* seems promising as an agent that interferes with Hp in colonized individuals. More studies are needed to confirm these results and to elucidate the mechanisms by which Sb inhibits Hp.

Key Words: *Children*, Helicobacter pylori, Saccharomyces boulardii, *prebiotic*, *probiotic*

Introduction

Helicobacter pylori (Hp) is a Gram-negative microorganism which colonizes the gastric mucosa and induces a chronic, asymptomatic gastritis in most people [1]. Hp is recognized as an aetiological agent for gastroduodenal ulcers and as a risk factor for the development of gastric lymphoma or adenocarcinoma [2,3]. Hp colonization occurs earlier, more intensely and more frequently in individuals from developing countries, who live in conditions of inadequate environmental hygiene, than in individuals from industrialized countries [4,5].

Antibiotic administration to asymptomatic children colonized by Hp is not recommended [6] and, in our experience, symptomatic children living in unsatisfactory hygienic conditions frequently relapse when treated (unpublished data). In addition, treatment has a high cost, it is associated with the appearance of bacterial resistance and it may also induce secondary phenomena such as diarrhoea, abdominal pain, allergy and taste disturbances. For these reasons, it is important to develop alternative forms of treatment to prevent or decrease Hp colonization in populations at risk. Thus, the use of probiotic microorganisms as a "possible" tool for the management of Hp infection appears attractive, as described in the report of the Maastricht 2000 Consensus Conference on *Helicobacter pylori* [7,8].

Indeed, some strains of probiotic lactobacilli have been shown to exert *in vitro* bactericidal activities against Hp and to inhibit Hp colonization to gastric cell lines and in animal models [9,10]. Such an anti-Hp activity has been confirmed in colonized adults and children [11–13] and, in the case of *Lactobacillus johnsonii* La1 (La1), it seems to increase with the

Correspondence: Martin Gotteland, Laboratory of Microbiology, INTA, University of Chile, El Libano 5524, Macul, Santiago, Chile. Tel: +56 2 6781523. Fax: +56 2 2214030. E-mail: mgottela@inta.cl

frequency of intake of the probiotic [14]. *Saccharomyces boulardii* (Sb), on the other hand, is a probiotic yeast widely used for prevention and/or treatment of diarrhoeal episodes, especially those due to relapses of enterocolitis associated with *Clostridium difficile* after antibiotic treatments [15,16]. Sb may be administered in synbiotic form together with fructo-oligosaccharides such as inulin, as it has been recently shown that this yeast uses these sugars as preferential substrates [17]. Currently, there are no studies supporting the possible protective effect of Sb against Hp.

On the basis of these findings, the main objective of this study was to evaluate the capacity of *Lactobacillus acidophilus* LB and of the synbiotic combination of Sb plus inulin to interfere with Hp colonization in children at the end of an 8-wk treatment.

Patients and methods

Subjects

The study was carried out in a school from a low socio-economic district in Santiago. The protocol was approved by the Ethics Committee of INTA. The inclusion criteria applied were: (1) children of either sex, 5 to 12 y of age; (2) written, informed approval by the parents, which specified that an antibiotic treatment was available for the children who were colonized at the end of this study, if requested; (3) absence of symptoms, particularly of those referred to the gastrointestinal tract; (4) a positive ^{13}C-UBT. The exclusion criteria were: (1) previously diagnosed gastrointestinal pathologies and (2) a history of antibiotic, antacid or prokinetic drug treatments in the previous month. The first ^{13}C-UBT screening was carried out to detect colonized children among 254 who satisfied the inclusion criteria; their mean age was 8.4 ± 1.6 y (range: 5 to 12 y).

To evaluate the possibility of spontaneous clearance of Hp, an additional 81 asymptomatic children (10.6 ± 2.4 y; range: 6 to 16 y), who also satisfied the requirements already mentioned and who were Hp-positive in a first ^{13}C-UBT, were followed for 8 wk without any treatment, when a second ^{13}C-UBT was carried out.

Study design

This was a randomised, open study. The 254 children positive for Hp by the ^{13}C-UBT were distributed using a random numbers table into three groups to receive (1) antibiotics (group Ab), or (2) *Lactobacillus acidophilus* LB (group LB), or (3) *Saccharomyces boulardii* plus inulin (group SbI). Treatments were administered daily at school every morning and afternoon for 8 wk. In order to ensure compliance with the study protocol, every child had to ingest the product in front of the field personnel; during weekends, antibiotics or products were sent home with the children for administration by the parents; any doses not ingested were returned to the investigators. Parents were asked to avoid giving their children commercial fermented dairy products throughout the duration of the study. Children absent from school for more than three consecutive days were discharged from the protocol. On the day after the end of the treatment, a second ^{13}C-UBT was performed to evaluate the level of gastric colonization.

Treatment and products

All antibiotics used in the study were purchased from Laboratorio Chile, Santiago, Chile. The antibiotic treatment, including lanzoprazole (1 mg/kg, b.i.d.), amoxicillin (50 mg/kg, t.i.d.) and clarythromycin (15 mg/kg, b.i.d.), was administered to the children from group Ab for 8 d. Children from group LB received a capsule containing 10^9 heat-killed and lyophilized LB (Lacteol Forte, Laboratoire du Dr. Boucard, Paris, France) b.i.d. for 8 wk. A sachet containing 250 mg of lyophilized Sb (Perenteryl, Merck Química Chilena, Santiago, Chile) was dissolved in an aqueous solution containing 5 g inulin (Orafti, Tienen, Belgium), and was also administered b.i.d. to the children from group SbI.

^{13}C-urea breath test

The ^{13}C-UBT was carried out after an overnight fast using a kit locally prepared for Hp detection. Children first drank a glass of orange juice to delay gastric emptying and afterwards a baseline breath sample was collected in duplicate by blowing with a straw into two Exetainers (Labco Ltd, High Wycombe, Bucks., UK). Seventy-five milligrams of ^{13}C-urea (Urea ^{13}C, pharmaceutical grade, 99% atom ^{13}C; Eurisotop, Gif-sur-Yvette, France) in water was then administered and a second breath sample was collected, also in duplicate, 30 min later. The tubes containing the breath samples were stored at room temperature until analysed. Breath samples were passed through a desiccant to eliminate water, and carbon dioxide was extracted by gas chromatography at 100°C under vacuum. The $^{13}C/^{12}C$ ratio in breath CO_2 was measured in a stable isotope ratio mass spectrometer (Breath-Mat Plus, Finnigan, Bremen, Germany), equipped with an autosampler, and compared with the Pee Dee Belemnite reference limestone standard. Baseline $^{13}CO_2$ levels measured before the administration of the labelled urea were subtracted from the 30-min values. Excess $\delta^{13}CO_2$ over baseline (DOB) values were expressed as parts per thousand and a breath test with DOB >5 at 30 min was considered positive for Hp. All children received a breakfast after the breath test.

Table I. Characteristics of the three groups of children.

	Ab	LB	SbI	
n	57	63	62	
Sex (%M)	45.6	58.3	44.3	n.s. ($\chi^2=2.89$)
Age (y)	8.4 ± 1.7	8.5 ± 1.7	8.3 ± 1.5	n.s.
DOB_1 (‰)				
Mean ± SD	34.3 ± 19.2	33.8 ± 16.4	34.9 ± 18.1	n.s.
Range	[5.2–93.4]	[6.2–90.9]	[5.3–98.0]	
Dropout rate	12 (21.0%)	17 (27.0%)	12 (19.3%)	n.s. ($\chi^2=2.14$)
n	45	46	50	
DOB_1 (‰)				
Mean ± SD	35.0 ± 21.1	32.7 ± 15.2	27.5 ± 18.2	n.s.
Range	[5.2–93.4]	[6.2–67.6]	[5.3–88.0]	
DOB_2 (‰)				
Mean ± SD	8.42 ± 13.23	33.39 ± 19.3	31.2 ± 17.4	(ANOVA: $F=30.8$; $p=0.000$)
Range	[1.6–63.5]	[0.21–84.3]	[−0.46–71.4]	

Statistics

Results were expressed as means ± SD or range. ΔDOB was expressed as means with 95% confidence intervals. Qualitative and quantitative variables were analysed by χ^2 and analysis of variance.

Results

Detection of children colonized by Hp was carried out by ^{13}C-UBT at the beginning of the study; 254 children were screened and Hp was detected in 182 of them (71.7%), with DOB1 values of 34.3 ± 17.8‰. These 182 children were finally randomized into the three groups whose characteristics are described in Table I. No significant differences between the groups were observed for age, sex and DOB1 values at the time of randomization. Of the 182 children randomized for treatment, 141 completed successfully both the treatment period and the second ^{13}C-UBT test (total study dropout: 22.5%). As shown in Table I, the distribution of the 41 dropouts was not significantly different with respect to group allocation ($\chi^2=2.14$, n.s.). Reasons for dropout were as follows: 21 subjects (51.2%) received other antibiotic treatments during the study; three (7.3%) were withdrawn by their parents; 12 (29.3%) missed school for more than three consecutive days; two (4.9%) left school because their families moved to another district; and three (7.3%) completed the treatment but did not return for the second breath test.

Hp eradication was successful in 66% (30/45) of the children from the Ab group, in 6.5% (3/46) of those of the LB group and in 12% (6/51) in those of the SbI group ($\chi^2=51.1$, $p<0.001$). The eradication rate was not significantly different between the two groups with probiotic treatments.

The differences between DOB values (ΔDOB) obtained before and after treatment are shown in Figure 1. A significant decrease in DOB values was observed in the antibiotic group (−26.6‰; 95%; CI: −33.9 to −19.3‰) and in the SbI group (−6.3‰; 95% CI: −6.3 to −0.8‰, i.e. a decrease of 16.8%) but not in the LB group (0.7‰; 95% CI: −5.8 to +7.2‰). The decrease induced by antibiotics was significantly greater than those induced by LB or SbI (ANOVA for the three groups: $F=18.97$, $p<0.000$); the decrease induced by SbI tended to be greater, but not significantly more so, than that induced by LB ($p=0.12$).

Of the 81 asymptomatic colonized children who were evaluated to assess the possibility of spontaneous clearance, 71 completed the study period (dropout rate: 12.3%) and all of them remained colonized after 8 wk, i.e. no spontaneous eradication was observed in these colonized children during this period.

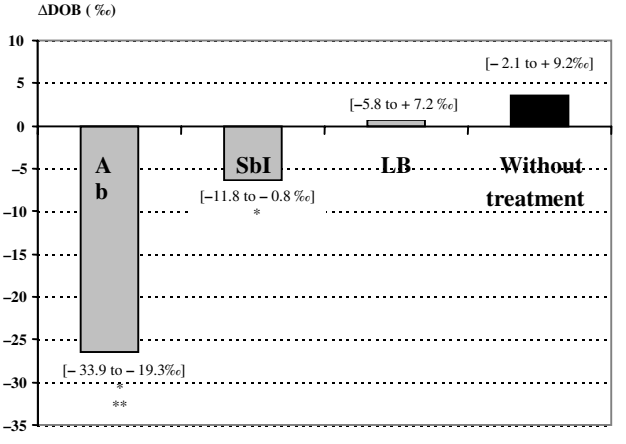

Figure 1. Differences (ΔDOB) between DOB values before and after treatment with antibiotics (Ab), *S. boulardii* + inulin (SbI) or *Lactobacillus* LB (LB) and in the group without treatment. Brackets indicate 95% CI of the differences in DOB values. Ab and SbI but not LB induced a significant decrease in DOB values (* $p<0.05$). The decrease induced by Ab was significantly higher than those induced by SbI and LB (** ANOVA: $F=18.97$, $p<0.000$).

Furthermore, no changes in DOB values were observed in these children, when comparing the results of their first (36.0 ± 17.4‰) and second ^{13}C-UBT (39.6 ± 25.3‰) (ΔDOB $= 3.6 \pm 23.9$‰; 95% CI: -2.1 to $+9.22$‰).

In a recent study carried out in children and adolescents using the same amount of ^{13}C-urea we used, Yang and Seo [18] observed a high frequency of false-positive results and low specificity in children 6 y of age or less; these authors calculated an optimal cut-off value of 4‰ in children older than 6 y and 7‰ in children 6 y of age or less. To validate our results taking into account these new cut-off values, we carried out an analysis of the DOB values obtained by us from our infected recruited children, treated and untreated. No false positive or false negatives were detected with these new cut-off values, supporting the validity of our results and conclusions.

Discussion

Our results show that about 72% of the children evaluated in this study were positive for Hp, confirming previously demonstrated high rates of colonization in the low socio-economic stratum living in Santiago [12,19]. This suggests that a high prevalence of Hp colonization persists in this population despite the considerable improvement of the sanitary conditions and nutrition observed in Chile during the last decades [20]. We recently observed that about 60% of Hp-positive children are colonized by virulent, *cag*A-positive strains in association with more severe forms of gastritis [21]. This is of particular importance because early gastric colonization and strain virulence are both considered long-term promoting factors for the development of gastric adenocarcinoma, a condition with a high prevalence in Chile [22]. These findings, in addition to the fact that asymptomatic children should not be treated with antibiotics, justify the need for exploring alternative forms of treatment capable of preventing, delaying and/or reducing infection rates in the paediatric population [7,8]. Some probiotic microorganisms, such as *Lactobacillus* strains, have been detected in the human stomach [23] where they may exert local protective effects [24,25]; these bacteria could represent an interesting alternative for competition with Hp colonization.

In this study we observed a decrease in DOB values after regular intake of SbI in children, a fact that is generally interpreted as resulting from decreases in urease activity in the stomach, reflecting interference with Hp. Different mechanisms may explain this effect of Sb. This probiotic yeast has been shown to stimulate the mucosal immune system, to exert trophic effects on the intestinal mucosa and to inhibit the *in vitro* growth of pathogens such as *E. coli*, *S. typhi*, *S. dysenteriae* and *C. albicans* [15]. Our results are interesting because, until now, there were no data available about the anti-Hp activity of Sb. We are demonstrating here for the first time that regular intake of Sb is capable of eradicating Hp in 12% of the colonized children. This could be due to a bactericidal effect on Hp or to effects on the toxins produced by Hp, as has been observed with other pathogens [26]. The other bacterial probiotic strain tested, LB, which is heat-killed and lyophilized, has been shown to interfere with Hp both *in vitro* and in animal models [9] and to increase Hp eradication rates during antibiotic treatments in human adults [13]. These findings, however, were not confirmed in our study as this strain seems to exert less effect than Sb against Hp colonization. This suggests that anti-Hp activity is species and strain specific, with some probiotics, such as Sb and La1 [12], interfering with Hp *in vivo* more actively, while others, such as LB, *L. paracasei* ST11 [12] or *Lactobacillus* GG (unpublished results), showed less or no activity.

To better interpret the results of the different treatments, it is important to know whether colonized children are able to clear Hp spontaneously. This was evaluated in 81 children from the same school who tested Hp positive with a first ^{13}C-UBT and were followed for 8 wk without any treatments. Interestingly, we observed that, in our setting, none of the subjects was able to clear Hp spontaneously. This is an important observation as this suggests that the eradication observed with SbI was not influenced by spontaneous clearance.

It is important to point out that, although the effects of SbI on the UBT results are statistically significant, the decrease observed is small and may not be clinically significant. However, a similar amplitude in the decrease of the ^{13}C-UBT values (5.7‰ of excess $^{13}CO_2$) has also been observed by Sakamoto et al. in colonized adults after 8 wk of administration of a yogurt containing *L. gasseri* (LG21) [27]; interestingly this decrease, though low, was also associated with a significant improvement of the serum ratio between pepsinogen I and pepsinogen II in these subjects as a reflection of the decrease of gastric inflammation. In our study, the mechanism by which inulin participates in the eradication of Hp is not clear, and obviously more research is required on this aspect.

In conclusion, *Saccharomyces boulardii* seems to be a promising tool that interferes with Hp in colonized subjects. It is necessary to carry out more clinical trials in children and adults to confirm these first observations; it is also necessary to explore the mechanisms that explain the protective effect of this probiotic.

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References

[1] Sachs G, Weeks DL, Melchers K, Scott DR. The gastric biology of *Helicobacter pylori*. Ann Rev Physiol 2003;65:349–69.

[2] NIH Consensus Development Panel on *Helicobacter pylori* in peptic ulcer disease. JAMA 1994;272:65–9.

[3] Kelley JR, Duggan J M. Gastric cancer epidemiology and risk factors. J Clin Epidemiol 2003;56:1–9.

[4] Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of *Helicobacter pylori* infection. Helicobacter 2004;9 Suppl 1:1–6.

[5] Hopkins RJ, Vial P, Ferreccio C, Ovalle J, Prado P, Sotomayor V, et al. Seroprevalence of *Helicobacter pylori* in Chile: vegetables may serve as one route of transmission. J Infec Dis 1993;168:222–6.

[6] Gold BD, Coletti RB, Abbott M, Czinn SJ, Elitsur Y, Hassall E, et al. *Helicobacter pylori* infection in children: recommendations for diagnosis and treatment. J Pediatr Gastroenterol Nutr 2000;31:490–7.

[7] Canducci F, Cremonini F, Armuzzi A, Di Caro S, Gabrielli M, Santarelli L, et al. Probiotics and *Helicobacter pylori* eradication. Digest Liver Dis 2002;34 Suppl 2:S81–3.

[8] Malfertheiner P, Megraud F, O'Morain C, Hungin AP, Jones R, Axon A, et al. Current concepts in the management of *Helicobacter pylori* infection. The Maastricht 2000 Consensus Report. Alim Pharmacol Ther 2002;16:167–80.

[9] Coconnier MH, Lievin V, Hemery E, Servin A. Antagonistic activity against *Helicobacter pylori in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. Appl Environ Microbiol 1998;64:4573–80.

[10] Kabir AMA, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. Gut 1997;41:49–55.

[11] Michetti P, Dorta G, Wiesel PH, Brassart D, Verdu E, Herranz M, et al. Effect of whey-based culture supernatant of *Lactobacillus acidophilus (johnsonii)* La1 on *Helicobacter pylori* infection in humans. Digestion 1999;60:203–9.

[12] Cruchet S, Obregón MC, Salazar G, Diaz E, Gotteland M. Effect of the ingestion of a dietary product containing *Lactobacillus johnsonni* La1 on *Helicobacter pylori* colonization in children. Nutrition 2003;19:716–21.

[13] Canducci F, Armuzzi A, Cremonini F, Cammarota G, Bartolozzi F, Pola P, et al. A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. Alim Pharmacol Ther 2000;14:1625–9.

[14] Gotteland M, Cruchet S. Suppresive effect of frequent ingestion of *Lactobacillus johnsonii* LA1 on *Helicobacter pylori* colonization in asymptomatic volunteers. J Antimicrob Chemother 2003;51:1317–9.

[15] Periti P, Tonelli F. Preclinical and clinical pharmacology of biotherapeutic agents: *Saccharomyces boulardii*. J Chemother 2001;13:473–93.

[16] Cremonini F, Di Caro S, Santarelli L, Gabrielli M, Candelli M, Nista EC, et al. Probiotics in antibiotic-associated diarrhoea. Digest Liver Dis 2002;34 Suppl 2:S78–80.

[17] Mitterdorfer G, Kneifel W, Vierntein H. Utilization of prebiotic carbohydrates by yeasts of therapeutic relevance. Lett Appl Microbiol 2001;33:251–5.

[18] Yang HR, Seo JK. Diagnostic accuracy of the ^{13}C-urea breath test in children: adjustment of the cut-off value according to age. J Gastroenterol Hepatol 2005;20:264–9.

[19] Vera JF, Gotteland M, Chavez E, Vial MT, Kakarieka E, Brunser O. Sucrose permeability in children with gastric damage and *Helicobacter* infection. J Pediatr Gastroenterol Nutr 1997;24:506–11.

[20] Albala C, Vio F. Epidemiological transition in Latin America: the case of Chile. Public Health 1995;109:431–42.

[21] Gotteland M, Corvalán A, Sarmiento F, Chávez E, Backhouse C, Palma M, et al. Gastric permeability is not increased in children colonized by *cag*A-positive or *cag*A-negative strains of *Helicobacter pylori*. Digest Liver Dis 2001;33:750–4.

[22] Llorens P. Gastric cancer in Chile. Gastrointest Endosc 1999;49:408–11.

[23] Marteau P, Minekus M, Havenaar R, Huis In't Veld JHJ. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. J Dairy Sci 1997;80:1031–7.

[24] Gotteland M, Cruchet S, Verbeke S. Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indomethacin in humans. Alim Pharmacol Ther 2001;15:11–7.

[25] Elliott SN, Buret A, McKnight W, Miller MJS, Wallace JL. Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. Am J Physiol 1998;38:G425–32.

[26] Pothoulakis C, Kelly CP, Joshi MA, Gao N, O'Keane CJ, Castaglioulo T, et al. *Saccharomyces boulardii* inhibits *Clostridium difficile* toxin A binding and enterotoxicity in rat ileum. Gastroenterology 1993;104:1108–15.

[27] Sakamoto I, Igarashi M, Kimura K, Takagi A, Miwa T, Koga Y. Suppressive effects of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in humans. J Antimicrob Chemother 2001;47:709–10.