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Use of Lactoferrin for *Helicobacter pylori* Eradication

Preliminary Results

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Abstract

Background: One-week triple therapy is the most frequently recommended treatment of *Helicobacter pylori* infection. The associated eradication rate is satisfactory; nevertheless, it is advisable to look for more effective therapies. Our aim was to test the efficacy of a standard triple therapy plus bovine lactoferrin for the eradication of *H. pylori* infection. **Study:** This open, randomized, single-center study was designed to include 150 consecutive *H. pylori*–positive patients with dyspeptic symptoms and gastritis who received triple therapy with rabeprazole, clarithromycin, and tinidazole plus lactoferrin for 7 days (group A), rabeprazole, clarithromycin, and tinidazole for 7 days (group B), or rabeprazole, clarithromycin, and tinidazole for 10 days (group C). *H. pylori* status was assessed 8 weeks after the end of treatment by means of the ^{13}C-urea breath test or *H. pylori* stool antigen test. **Results:** The 7-day treatment including lactoferrin (group A) was successful in 100% (24/24) of the patients. The eradication rates in groups B and C were 76.9% (20/26 patients; 95% CI, 61%–93%) and 70.8% (17/24 patients; 95% CI, 53%–89%), respectively. A significant difference was found between group A and group B ($P = 0.023$) and group A and group C ($P = 0.022$). No differences were found between group B and group C ($P = 1.00$). **Conclusion:** These results suggest that lactoferrin could be a new, effective agent when added to antimicrobial therapy for the eradication of *H. pylori*. This treatment schedule could be proposed for larger trials of *H. pylori* eradication therapy, focusing on the excellent preliminary cure rate, good compliance to the treatment schedule, and relatively low price of lactoferrin for full treatment.

Key Words: Lactoferrin—*Helicobacter pylori*—Eradication—Therapy.

It is well established that 7-day triple therapy is associated with rates of *Helicobacter pylori* eradication ranging from 90% to 95%.[1,2] Gastroenterologists and microbiologists are continuously searching for new therapeutic approaches because of the increasing number of subjects who may be infected with *H. pylori* and the large burden of drugs for second-line therapy.[3] Consequently, bovine lactoferrin (bLf) has been suggested as an addition to the well-established 1-week proton pump inhibitor–based triple therapy (rabeprazole, 20 mg B.I.D.; clarithromycin, 500 mg B.I.D.; and tinidazole, 500 mg B.I.D.) for *H. pylori* eradication. bLf is a protein consisting of a single polypeptide chain. It weighs between 75 and 80 kd and transports two iron atoms in human and bovine milk.[4,5] Moreover, bLf has been demonstrated to have bacteriostatic and bactericidal activities against various infectious agents,[6,7] and further antibiotic effects of bLf have been attributed to its ability to bind to iron with great affinity, preventing iron utilization by *H. pylori* for growth.[8,9] bLf is also contained in the specific granules of polymorphonuclear leukocytes, and it is released from them when they are activated.[7] Furthermore, bLf appears to cause harmful changes to bacterial membrane permeability and the release of lipopolysaccharide from the outer membrane, with many protective immunologic events.[10] In this study, we investigated the contribution of bLf, at a dosage of 200 mg B.I.D. (100-mg capsules), to first-line 1-week triple therapy for *H. pylori* eradication.

METHODS

This open, randomized, single-center study was designed to include 150 consecutive *H. pylori*–positive patients with dyspeptic symptoms and gastritis. The presence of *H. pylori* infection was assessed at baseline by histologic analysis plus the ^{13}C-urea breath test or *H. pylori* stool antigen test (Meridian Diagnostics, Milan, Italy). For histopatologic examination, five biopsy specimens (2 at the antrum, 1 from the incisure, and 2 from the body) were obtained. Biopsy specimens were oriented and embedded in paraffin. Sections were treated with hematoxylin–eosin stain for histologic evaluation of gastritis and with May-Grünwald–Giemsa stain for identification of *H. pylori*. Patients were not treated with acid inhibitors or antibiotics in the 3 weeks before testing for *H. pylori* status. Eight weeks after completion of treatment, all patients un-

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derwent the ^{13}C-urea breath test (n = 44) or *H. pylori* stool antigen test (n = 28).

Preliminary statistical analysis was performed when one half of the patients (n = 74) were enrolled for *H. pylori* eradication therapy. The patients were randomized to the following three treatment groups: group A, rabeprazole (20 mg B.I.D.), clarithromycin (500 mg B.I.D.), and tinidazole (500 mg B.I.D.) plus bLf (200 mg B.I.D.; Dicofarm, Rome, Italy) for 7 days; group B, rabeprazole (20 mg B.I.D.), clarithromycin (500 mg B.I.D.), and tinidazole (500 mg B.I.D.) for 7 days; or group C, rabeprazole (20 mg B.I.D.), clarithromycin (500 mg B.I.D.), and tinidazole (500 mg B.I.D.) for 10 days. This was the only interim analysis performed in our study that was not prespecified. The content of lactoferrin per capsule is standardized in the manufacturing process; each capsule contained 100 mg of bLf.

Exclusion criteria were as follows: history of definitive acid lowering surgery, reflux esophagitis of greater than stage A, previous esophageal surgery, treatment with proton pump inhibitors within the last 2 weeks, or treatment with any antibiotics within the last 4 weeks before study enrollment. Pregnant or lactating women or patients with a proven allergy to clarithromycin or benzimidazole, a history of eradication therapy, renal or hepatic chronic diseases, and neoplasm were excluded from the study. All criteria were assessed by means of a complete history, physical examination, endoscopy, histologic examination, and analysis of biochemical blood samples. We did not test for *H. pylori* resistance to clarithromycin because of the very low level of primary resistance (<2%) recently demonstrated in our region.[11]

The study was performed according to the declaration of Helsinki, and all patients included in the study gave oral informed consent at enrollment. In addition, written instructions, including general explanations concerning the rationale and importance of an accurate intake of medication, a detailed description of the treatment, and a daily table of drug consumption, were given to the patients. Compliance was evaluated by pill count, and side effects were recorded by means of a structured clinical interview immediately after the end of the treatment schedule.

Fisher exact test was used for 2×2 tables.[12] A P value of <0.05 was considered statistically significant. An intention-to-treat approach was used. For all calculations, Bio Medical Data Processing (BMDP Dynamic version 7; University of California, Los Angeles, CA) was used.

RESULTS

Of 74 patients enrolled in the study, 17 had peptic ulcer, and 57 had gastritis. There were no statistically significant differences among the three groups in terms of the male-to-female ratio, smoking habits, and alcohol consumption. All but two patients completed treatment with good compliance (95% of patients took >90% of the pills). Two patients in group C withdrew from the study because of side events (dizziness, headache, and disgust). A statistically significant difference was found between group A and group B ($P = 0.023$) and between group A and group C ($P = 0.022$). No differences were found between group B and group C ($P = 1.00$). The findings for the three groups are summarized in Table 1. The ^{13}C-urea breath test or *H. pylori* stool antigen test revealed that all but 13 patients were negative for *H. pylori* at the end of the follow-up (2 months after the end of therapy). Six patients had mild adverse events (fatigue, dizziness, headache, diarrhea, tiredness, bitter taste, and skin rash); however, they completed the eradication therapy schedule with spontaneously disappearance of symptoms.

TABLE 1. *Findings for three groups*

	Group A	Group B	Group C
Intention-to-treat	24/24 (100%)	20/26 (76.9%)	17/24 (70.8%)
95% CI	—	61–93	53–89

DISCUSSION

This preliminary analysis demonstrated a 100% eradication rate for 7-day triple therapy with rabeprazole (20 mg B.I.D.), clarithromycin (500 mg B.I.D.), and tinidazole (500 mg B.I.D.) plus bLf (200 mg B.I.D.) compared with 76.9% for the "classical" 7-day therapy and 70.8% for the same schedule for 10 days. It is well known that addition of a proton pump inhibitor increases both stability and activity of antibiotics, promotes a peculiar activity against *H. pylori*, and inhibits urease activity of the bacterium. As with other proton pump inhibitors, rabeprazole has in vitro antibacterial activity against *H. pylori*; its activity against this organism is greater than that of either lansoprazole or omeprazole.[13] By using different points of attack against the bacterium, clarithromycin, tinidazole, and bLf exert optimal activity against *H. pylori* that could lead to complete destruction of the structure of the bacterium. Several in vivo and in vitro studies of the effects of bLf on *H. pylori* infection did not show univocal results.[9,15] A recent study assessing the safety and efficacy of recombinant human lactoferrin as monotherapy for *H. pylori* infection in adult subjects showed no beneficial effect regarding the elimination of the bacterium[14]; possibly, the results could be different with more subjects, with more long-lasting therapy, or with concomitant acid-suppressing therapy. It is possible to speculate that bLf in association with antibiotics and proton pump inhibitors may increase the effectiveness and diminish the side events associated with the current eradication treatment schedule.

Our preliminary results raise the possibility of bLf being used in addition to antimicrobials in *H. pylori* eradication treatment, even if further data are needed. This treatment schedule, therefore, could be proposed for larger trials of *H. pylori* eradication therapy, focusing on the excellent cure rate (100% in the preliminary analysis), low rate of minor side effects, good compliance to the treatment schedule, and low price of bLf (13 Euro) for full treatment.

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