Bovine lactoferrin for *Helicobacter pylori* eradication: an open, randomized, multicentre study

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SUMMARY

Background

Cure rates for eradication of *Helicobacter pylori* appear to be decreasing, thus more effective therapies must be identified.

Aim

To evaluate the efficacy of bovine lactoferrin in the treatment of *H. pylori* infection.

Methods

In a multicentred prospective study, 402 (mean age 52.4, range 19–84 years) *H. pylori*-positive patients were assigned to one of three regimens: group A – esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days; group B – lactoferrin 200 mg b.d. for 7 days followed by the same schedule of group A; group C – esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. plus lactoferrin 200 mg b.d. for 7 days.

Results

Of the 402 patients, 389 completed the study. Six patients were discontinued due to side effects, one patient in group B died and six patients were lost to follow up. The eradication rate (intention-to-treat analysis) was 77% in group A (105/136), 73% in group B (97/132) and 90% in group C (120/134) (χ^2-test $P < 0.01$). The incidence of side effects was 9.5% in group A, 9% in group B and 8.2% in group C (χ^2-test $P = 0.1$).

Conclusion

This study demonstrates that bovine lactoferrin is an effective adjuvant to 7-day triple therapy for eradication of *H. pylori* infection.

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INTRODUCTION

According to the Maastricht consensus, the first-line therapy for *Helicobacter pylori* eradication is a combination of proton pump inhibitors or ranitidine bismuth citrate and clarithromycin plus either amoxicillin or metronidazole.[1] In first-line therapies, eradication rates using combinations of proton pump inhibitor-based triple therapies range from 75% to 98%, with most of them near 80%.[2–7] This signifies that up to 20% of patients are expected to be treatment failures, a value which, as already observed,[8] could be even higher in areas with a high prevalence of resistant *H. pylori* strains. Reasons for treatment failure are various and include poor compliance, treatment duration, number and dose of the prescribed drugs and bacterial resistance to antibiotics.[9] While culture and susceptibility tests are sensitive in refractory patients, the results observed *in vivo* are often disappointing when tailored to reflect *in vitro* susceptibility to standard anti-*Helicobacter* antibiotics.[10]

Gastroenterologists and microbiologists continue the search for new therapies due to the increasing number of target subjects for *H. pylori* and to the physiological and pharmacoeconomic burden of a second course of therapy.[11, 12] Bovine lactoferrin (bLf) is a single polypeptide chain glycoprotein of the transferrin family weighing between 75 000 and 80 000 Da. It transports two iron atoms with high affinity, even in an acidic environment[13, 14] and is present in milk, saliva, tears, bile, blood plasma and mucosal and genital secretions.[15] bLf has been shown to be bacteriostatic and bactericidic against various infectious agents.[16, 17] Additional antibiotic activity has been attributed to its ability to bind iron with great affinity, preventing iron utilization by *H. pylori*[18, 19] for growth; finally, lactoferrin has also been shown to inhibit the attachment of *H. pylori* to gastric epithelial cells.[20]

To evaluate its efficacy when added to triple therapies against *H. pylori*, a dosage of 200 mg daily bLf (capsules of 100 mg, b.d.) was added to well-established 1-week triple therapy regimens as first-line treatment. The efficacy of bLf was also evaluated when administered before a standard triple therapy; in fact, bLf with its well-known properties in diminishing bacterial load and activity of *H. pylori*,[16–19] could facilitate the action of antibiotics against the bacteria.

MATERIALS AND METHODS

The study was conducted from June 2003 to December 2004 as a prospective, open-labelled, multicentred trial with the participation of 14 centres in Italy.

The subject exclusion criteria were as follows: (i) previous *H. pylori* eradication therapy; (ii) history of definitive acid lowering surgery; (iii) reflux oesophagitis >A; (iv) previous oesophageal surgery; (v) treatment with proton pump inhibitors within the last 2 weeks or any antibiotics within the last 4 weeks before study enrolment; (vi) patients with a proven allergy to clarithromycin or benzimidazole; (vii) pregnant or lactating women; (viii) subjects with renal or hepatic chronic diseases; and (ix) subjects with any neoplasm. All criteria were assessed by means of a complete history, physical examination, endoscopy, histology and analysis of biochemical blood samples. *H. pylori* clarithromycin resistance was not performed because of the very low level of primary resistance (<2%) recently demonstrated in our region.[21]

The study was performed according to the principles of good clinical research practice, the Declaration of Helsinki and all patients included in the study had given informed consent on entering the study. Written instructions, including general information concerning the study rationale and objectives, the importance of compliance, a detailed description of the treatment and a daily written schedule of drug consumption were given to the patients. Compliance and side effects were recorded during a structured clinical interview immediately after completion of the drug regimen or at any time if required. Study subjects were considered to be non-compliant if <90% of study medication was taken.

A total of 402 consecutive *H. pylori*-positive patients suffering from dyspeptic symptoms, gastritis and peptic ulcer disease were randomized in a 1:1:1 ratio into the three treatment groups. The presence of bacterium was assessed at baseline by histology and the C^{13}-Urea breath test (C^{13} UBT) or histology and the *H. pylori* stool antigen-test (HpSA) (Meridian Diagnostics, Milan, Italy). In all centres, the C^{13} UBT was considered positive if the δ-value from baseline to 30 min (that is, the algebraic difference between the δ-value at 30 min and baseline) was $\geq 5\permil$.

For histopathology, five biopsies (two from the antrum, one from the incisure and two from the body) were taken. Biopsies were fixed and embedded in

paraffin. Sections were stained with haematoxylin and eosin for the histological evaluation of gastritis and with May Grünwald–Giemsa for identification of *H. pylori*. According to the recommendations of Maastricht 2 consensus for the monitoring of *H. pylori* eradication,[1] 8–10 weeks after completion of therapy, all subjects voluntarily underwent *H. pylori* testing with the C^{13} UBT or HpSA. Patients were not taking acid inhibitors or antibiotics in the 3 weeks before the final testing for *H. pylori* status. Failure of eradication therapy was defined as a positive breath test or HpSA.

According to the randomization list, patients were assigned to one of the three regimens: esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days (group A); bLf (Dicofarm, Roma, Italy) 200 mg b.d. for 7 days followed by esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days (group B) or esomeprazole 20 mg b.d., clarithromycin 500 mg b.d., tinidazole 500 mg b.d. plus bLf 200 mg b.d. for 7 days (group C). Each bLf capsule contained 100 mg of bovine lactoferrin. Subjects were instructed to take the proton pump inhibitor before breakfast and dinner, and all antibiotics and bLF after these meals.

Statistics

Data were analysed by a masked statistician (G.L). Frequency tables were analysed by means of the Pearson chi-square with standardized deviations. For all other variables, the Fisher's exact test and *t*-test were used as appropriate.

Both per protocol analysis (PP) (based on patients who completed the study) and intention-to-treat analysis (ITT) (a restrictive analysis that considers all drop-outs as 'failures' of the given treatment) were performed. A *P*-value less than 0.05 was considered statistically significant.

For all calculations, Bio Medical Data Processing (BMDP, Dynamic version 7; University of California, Los Angeles, CA, USA) was used.

RESULTS

Of the 402 patients, 389 were fully compliant to the protocol (more than 95% of study drugs taken). Some major side effects (diarrhoea and rush) led to treatment discontinuation in six patients (two in each group). One patient in group B died because of a street incident, while six patients (three in group A, two in group B and one in group C) were lost to follow-up. Of the 402 patients (210 men, 192 women with a mean age of 52.4, range 19–84 years) enrolled in the study, 102 had peptic ulcer and 300 had gastritis.

No statistically significant differences were observed among the three groups for diagnosis, male/female ratio, smoking habits and alcohol consumption (see Table 1). Specifically, group A was comprised of 136 subjects (70 male, 66 female), with a mean age of

Table 1. Baseline demographic and clinical characteristics of a total of 402 *Helicobacter pylori*-positive subjects with peptic ulcer or gastritis treated for the first time with one of three first-line regimens described below for eradication of *H. pylori*

	Group A*	Group B†	Group C‡
Number of patients	136	132	134
Median age (±S.D.) (years)	50.1 ± 11.1	50.5 ± 15.3	52.3 ± 15.8
Female/male	66/70	60/72	66/68
Smoking, *n* (%)			
Yes	36 (26.4)	27 (20.4)	35 (26.1)
No	136 (73.6)	132 (79.6)	134 (73.9)
Alcohol intake (%)			
<40 g/day	48	36	30
>40 g/day	8	11	16
Peptic ulcer	36	30	36
Gastritis	100	102	98

* Esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days.
† Bovine lactoferrin (bLf) 200 mg b.d. for 7 days followed by esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days.
‡ Esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. and bLf 200 mg b.d. for 7 days.

50.1 ± 11.1 years: 100 (74%) cases of gastritis and 36 (26%) cases of ulcer disease. Group B was comprised of 132 subjects (72 male, 60 female), with a mean age of 50.5 ± 15.3 years: 102 (77%) cases of gastritis and 30 (23%) cases of ulcer disease. Group C was comprised of 134 subjects (68 male, 66 female), with a mean age of 52.3 ± 15.8 years: 98 (73%) cases of gastritis and 36 (27%) cases of ulcer disease.

All patients but 67 were negative to C^{13} UBT or HpSA at the end of the follow-up period (2 months after completion of therapy). Eradication rates in group A were 77.2% (105/136) using an ITT analysis of all subjects and 78.3% (105/134) using a PP analysis conducted after excluding uncompleted subjects. In group B, eradication rates were 73.4% (97/132) using an ITT analysis and 76.9% (97/126) using a PP analysis. Eradication rates in group C were 89.5% (120/134) using an ITT analysis and 93% (120/129) using a PP analysis. Results for the three groups are summarized in Table 2.

Both ITT and PP analyses showed that the eradication rate was significantly higher in group C compared to the other two regimens ($P = 0.01$, ITT analysis; $P = 0.001$, PP analysis). Smoking habits, alcohol intake, clinical presentation and diagnostic tests used (C^{13}UBT/HpSA) did not influence eradication rates (P = N.S. based on ITT analysis). The incidence of mild side effects (fatigue, dizziness, headache, diarrhoea, tiredness, bitter taste and skin rash) that occurred during therapy was 9.5% (13/136) in group A, 9% (12/132) in group B and 6.7% (9/134) in group C (χ^2-test P = N.S.). These symptoms spontaneously disappeared when patients completed the eradication schedule.

DISCUSSION

In this prospective, multicentred randomized study, the addition of bLf to the standard 1-week triple therapy for *H. pylori* infection significantly increased its eradication rate with respect to that of the non-supplemented regimen using both ITT and PP analyses. Conversely, a 1-week administration of bLf previous to the 1 week of standard triple therapy (group B) did not improve eradication rates and were comparable to those previously reported with standard triple therapy regimens.

Optimizing *H. pylori* eradication therapy remains an ongoing challenge worldwide. Although a great deal of research has focused on treatment of *H. pylori* since the discovery of its crucial role in gastrointestinal disease, currently up to 25% of patients enrolled in clinical trials are treatment failures, even using the widely accepted and efficacious regimens that have gained the imprimatur of consensus guidelines.[22] A disappointing cure rate of <80% after a 7-day triple therapy was again confirmed in the present study.

Among the new options brought to light recently in the armamentarium against *H. pylori*, bLf was chosen in the present study for several reasons: (i) its antimicrobial activity against *Helicobacter* species *in vitro* and *in vivo* and effectiveness at inhibiting its growth at pH 6;[19] (ii) antibiotic activity attributed to its ability to bind iron with great affinity and prevent its utilization by bacteria; in fact, deferoxamine, another iron chelator, inhibits *H. pylori* growth;[18, 23, 24] (iii) a non-iron dependent activity against *H. pylori* not yet identified;[19] (iv) other actions on the gastric mucosa that might reduce stomach size in an infection, such as an

Table 2. From a total of 402 *Helicobacter pylori*-positive subjects with peptic ulcer or gastritis, the number of subjects successfully eradicated after first-line therapy with one of three drug regimens described below is shown

	Group A*	Group B†	Group C‡	P
PP [95% CI]	105/134 (78.3%) [71–85]	97/126 (76.9%) [84–69]	120/129 (93%) [97–88]	0.001
ITT [95% CI]	105/136 (77.2%) [69–85]	97/132 (73.4%) [65–81]	120/134 (89.5%) [95–85]	<0.01
Dropouts	2	6	5	N.S.
Side effects	13/136 (9.5%)	12/132 (9%)	9/134 (6.7%)	N.S.

N.S., not significant; PP, per protocol analysis; ITT, intention-to-treat analysis.

* Esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days.

† Bovine lactoferrin (bLf) 200 mg b.d. for 7 days followed by esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. for 7 days.

‡ Esomeprazole 20 mg b.d., clarithromycin 500 mg b.d. and tinidazole 500 mg b.d. and bLf 200 mg b.d. for 7 days.

immune-modulatory activity;[18, 23, 25] (v) a proposed antioxidant activity;[26, 27] and (vi) a significant inhibitory effect on the *in vivo* attachment of *H. pylori* to the stomach, associated with a reduction in bacterial number and associated inflammation.[14] A number of other potential mechanisms by which lactoferrin inhibits the growth of several microorganisms has been suggested, including structural changes in the microbial cell wall, complete loss of membrane potential and integrity, indirect effects on enzyme activation, an increased generation of metabolic by-products of aerobic metabolism, iron deprivation and a combination of these factors.[28–32]

By using multiple points of attack against the bacterium, bLf plus clarithromycin and tinidazole may exert an optimal synergic anti-*H. pylori* activity that could lead to a complete destruction of the bacterium. Several *in vivo* and *in vitro* studies regarding a monotherapy of bLF against *H. pylori* infection showed equivocal results. A recent study that assessed the clinical safety and efficacy of a recombinant human lactoferrin (hLf) monotherapy for *H. pylori* infection in adult subjects did not show significant eradication;[33] however, the authors suggested that the lack of effect was due to the limited number of subjects, and that as an adjuvant to long-lasting therapy or with concomitant acid-suppressing therapy it might have been more effective. Nevertheless, it is controversial whether hLf is effective against *H. pylori*, particularly since it is known to be a growth-supporting iron transport protein for bacteria, whereas bLf is not.[34] In fact, more concrete data is available about the anti-*H. pylori* efficacy of bLf than about hLf. In our previously published trials on the efficacy of bovine lactoferrin in curing *H. pylori* infection,[35, 36], the addition of bLf to a 7-day clarithromycin-tinidazole-rabeprazole therapy significantly increased eradication rates compared to the same antibiotic combination without bLF (92.2% vs. 71.2% at ITT analysis). Conversely, a recent study evaluating whether addition of bLF could improve the efficacy of a clarithromycin–amoxicillin based triple therapy as first-line treatment for *H. pylori* infection in non-ulcer dyspepsia patients was disappointing.[37] It is possible that bLF and tinidazole exert a synergistic effect against the bacteria, while bLF and amoxicillin do not.

Guidelines often suggest that an acceptable success rate for a particular therapy against *H. pylori* infection should be above 80% on an intention-to-treat basis.[1] In the present multicentred study, both ITT and PP analyses showed that the eradication rate was higher than 89% for patients in the bLf adjuvant group, results that confirmed those in previous studies.[35, 36]. Interestingly, a pre-treatment with bLF as monotherapy for 7 days (group B) did not provide any additive therapeutic effect to a subsequent standard 7-day triple therapy, suggesting that bLf might potentiate the activity of the co-administered antimicrobials in the eradication of *H. pylori*, producing a combination that maximizes antibiotic activity, minimizes dosage and potentially lessens the side effects of the other commonly used antibiotics. In conclusion, adjuvant therapy with bLF, in conjunction with a standard triple therapy of esomeprazole, clarithromycin and tinidazole, provided satisfactory eradication rates and therefore, could be proposed as a valid alternative regimen in *H. pylori* eradication aimed at a high cure rate (89.5%), a low rate of minor side effects and good compliance.

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