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Human recombinant lactoferrin is ineffective in the treatment of human Helicobacter pylori *infection*

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SUMMARY

Background: Lactoferrin, a multifunctional glycoprotein, is known to have anti-microbial actions. Bovine lactoferrin and recombinant human lactoferrin have been shown to inhibit *Helicobacter pylori*, and more recently recombinant human lactoferrin was found to significantly increase the eradication rate of *H. pylori* when added to standard triple therapy.

Aim: To determine the efficacy, safety and tolerability of recombinant human lactoferrin as a therapy in suppressing or eliminating *H. pylori* infection in subjects with minimal upper gastrointestinal symptoms who have not previously been treated.

Subjects and methods: Nine healthy subjects with minimal upper gastrointestinal symptoms and a positive urea breath test were recruited. None of the volunteers had previously been treated for *H. pylori*. Subjects received 5×1.0 g human recombinant lactoferrin daily for 5 or 14 days. Breath tests were repeated during therapy and shortly after to check for eradication. The safety and tolerability of the drug were assessed by physical examination, by monitoring adverse events, and clinical laboratory evaluation.

Results: No conversion of the urea breath test from positive to negative was observed and there was no consistent change in urea breath test count to indicate a possible suppression of *H. pylori*.

Conclusion: Lactoferrin, given as a single agent, does not eradicate *H. pylori* infection.

INTRODUCTION

Lactoferrin is a multifunctional iron-binding glycoprotein which is found in human milk and in several mucosal secretions, e.g. saliva, tears, bile, pancreatic and seminal fluid, as well as specific granules of polymorphonuclear leukocytes.[1] Although lactoferrin has been isolated and characterized for many years,[2] knowledge of its biological functions remains limited. Over the last two decades, lactoferrin has been recognized to be an important factor in host defence against a wide range of bacteria.[3, 4]

Different mechanisms of antibacterial effects have been described. As an iron-binding protein, which is mostly secreted in its iron deficient form, lactoferrin limits the amount of free iron available. Iron is an essential growth factor for micro-organisms and by its deprivation, metabolic activities are limited.[5] Furthermore, lactoferrin has been found to destabilize the outer membrane of Gram-negative bacteria[6] and the peptides that result from proteolytic cleavage of lactoferrin seem to be more bactericidal than lactoferrin itself.[7] Another mechanism has been demonstrated in bovine lactoferrin—it inhibits bacterial attachment to epithelial cells.[8] Findings suggest that lactoferrin

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may play an anti-inflammatory role by modulating activation of the complement system.[9] It appears to act as a negative feedback mechanism to prevent the recruitment and activation of more leukocytes in sites of inflammation, after release from activated neutrophils.[10]

Helicobacter pylori, as the Gram-negative bacterium that causes chronic gastritis, peptic ulcer diseases and is involved in the genesis of gastric cancer, seems to be acquiring increasing resistance to commonly used antimicrobial agents. In the search for new agents, lactoferrin has been investigated in the past.

In vitro[11] and *in vivo* mice models[8, 12] confirmed the action of bovine and recombinant human lactoferrin against *H. pylori.*

A human trial with orally administered recombinant lactoferrin over a period of 24 h showed no effect of lactoferrin in eradicating *H. pylori* at either a low or high dose. Of six subjects receiving 5×250 mg of recombinant human lactoferrin and six subjects receiving 5×1 g over a 24-h period, none showed a conversion of the urea breath test (UBT) result from positive to negative.[13]

However, in a recent trial, bovine lactoferrin was added to a standard triple eradication therapy regime (rabeprazole, clarithromycin and tinidazole) and a significant improvement in eradication rate from 78% to 100% was achieved.[14]

After the promising results using lactoferrin as an adjunctive agent and its demonstrated anti-microbial effects, both *in vitro* and in animal models, the aim of this study was to observe the activity of lactoferrin *in vivo*. Lactoferrin as a single agent, in higher doses and over a longer treatment period than previously, was investigated for its activity against *H. pylori*.

METHODS

Study design and subjects

Subjects were recruited from medical and administrative staff employed at the Sir Charles Gairdner Hospital, Perth, Australia. Healthy volunteers were sought with minimal upper gastrointestinal discomfort. The presence of *H. pylori* infection was determined by urea breath test and a strongly positive result of > 500 disintegrations per min was used as the criterion of infection. A total of nine suitable volunteers between the ages of 22 and 53 years were recruited after the screening of 92 subjects. Six subjects received 1.0 g lactoferrin five times daily for a period of 5 days and three subjects received the same dose of lactoferrin over a period of 14 days.

The safety and tolerability of the drug were assessed by physical examination and clinical laboratory evaluation (urea and electrolytes, full blood count, urinalysis, serum iron, ferritin, β-human chorionic gonadotropin (β-HCG)) before and after treatment, as well as monitoring of adverse events. The laboratory data and physical examination of all subjects were within normal limits prior to treatment.

Originally the recruitment of 12 subjects was planned, but as the outcome of the study became obvious, recruitment was ceased after nine volunteers had finished treatment.

The Human Research Ethics Committee of the Sir Charles Gairdner Hospital approved the study.

Recombinant human lactoferrin

Recombinant human lactoferrin was produced through a fermentation process employing genetically modified *Aspergillus niger* var. *awamori* by Agennix Incorporated, Houston, Texas. The recombinant protein was shown to be indistinguishable from native breast milk lactoferrin in terms of size, immunoreactivity, iron binding, receptor binding and anti-microbial properties.[15]

^{14}C-urea breath test

A ^{14}C-urea breath test was used (PYtest, Tri-Med Distributors Pty Ltd, Western Australia), which includes a capsule containing 37 kBq of ^{14}C-urea. The fasting subject swallowed a capsule and delivered a breath sample 10–15 min later. The breath sample was analysed at the Tri-Med laboratories. UBT analysis was carried out prior to starting lactoferrin therapy (day 0), during therapy (day 3, in a 5-day therapy course, or day 8, in a 14-day therapy course) and after completing therapy (days 6 or 15).

RESULTS

After receiving 1.0 g lactoferrin five times daily for a period of 5 ($n = 6$) or 14 days ($n = 3$), respectively, no significant adverse effects were observed (one subject complained of flatulence and dysmenorrhoea, one

subject complained of abdominal cramps). No changes in routine laboratory results were observed during or after medication intake.

None of the nine volunteers were cured of their *H. pylori* infection. UBT results were persistently positive (Figure 1, Table 1). There was at least an initial decrease in the UBT counts in five patients. Three patients showed a further decrease after the cessation of treatment. There was no significant trend formation in the individual patient data.

DISCUSSION

In view of the development of increasing resistance not only of *H. pylori* against anti-microbial agents, but also the accompanying often-deleterious side-effects of anti-biotic treatment, a natural substance with no relevant side-effects such as lactoferrin would be a welcome treatment alternative or addition.

Earlier work reported that lactoferrin might serve as a source of iron for *H. pylori*, and that this iron acquisition

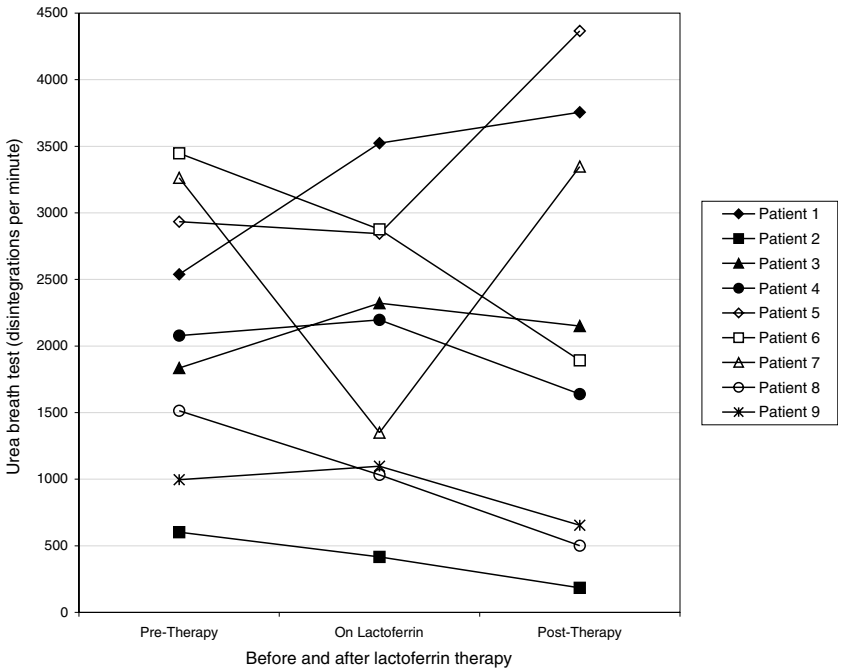

Figure 1. Effect of lactoferrin on the urea breath test counts of *H. pylori* positive subjects.

Table 1. Number, age and sex of participating subjects and measured urea breath test (UBT) counts in disintegrations per minute (d.p.m.)

Patient number	Age (years)	Sex	UBT				
			pre-Rx	Day 3	Day 6	Day 8	Day 15
1	50	F	2538	3523	3755	—	—
2	22	F	602	417	184	—	—
3	38	F	1835	2322	2150	—	—
4	25	F	2079	2197	1639	—	—
5	37	M	2934	2844	4366	—	—
6	27	M	3447	2876	1893	—	—
7	53	F	3263	—	—	1350	3348
8	44	F	1514	—	—	1033	501
9	29	F	996	—	—	1097	654

An initial group of six subjects received 5 days of lactoferrin treatment and were tested after days 3 and 6; a second group of three subjects received 14 days of lactoferrin treatment and the UBT count was measured on days 8 and 15.

by *H. pylori* would stimulate the bacterial growth[16] and aid stomach colonization. *H. pylori*-positive patients with gastritis have been found to have significantly higher lactoferrin concentrations in gastric juice than those without infection,[17] and the eradication of *H. pylori* significantly reduced levels of lactoferrin in gastric mucosa.[18]

However there was no eradication of *H. pylori* observed after a 24 h treatment period with lactoferrin as a single treatment agent,[13] consistent with the findings in the present trial, when lactoferrin was given for a longer duration and in higher doses. No suppressive or beneficial effect regarding the elimination of *H. pylori* infection could be found.

In contrast to the *in vivo* studies, *in vitro* experiments have shown *Escherichia coli* strains to be sensitive to lactoferrin, with the replication of bacteria almost completely prevented by lactoferrin alone.[5]

The increased sensitivity of bacteria to antibiotics such as vancomycin, rifampicin, doxycycline and chloramphenicol, in the presence of lactoferrin, has been reported.[19–21] As there was no bactericidal or bacteriostatic effect seen by lactoferrin on its own[22] the main role of lactoferrin may be to enhance the effectiveness of the anti-microbial therapy. One of the postulates about the possible mechanism of lactoferrin is that the cationic lactoferrin binds to the anionic cell wall materials to allow a greater penetration of the antibiotic.[16]

A recent preliminary study has shown a possible potential effect of lactoferrin on the *H. pylori* eradication rate in combination with standard triple therapy.[20] However, this has yet to be confirmed in further trials, since the authors did not determine the antibiotic susceptibility of the *Helicobacter* strains in each patient prior to therapy.

If the individual data of the present trial are compared to each other, no tendency of trend formation towards a general decreased UBT count could be observed. However, it is still controversial whether the results of ^{13}C- and ^{14}C-UBT may be usefully employed to semiquantitatively assess the intragastric bacterial load,[23, 24] or if the UBT should be used only as a qualitative test[25] to determine if a patient's eradication therapy was successful.[26] Clearly there is a need to develop a better way to non-invasively quantify *H. pylori* infection.

Lactoferrin is currently considered to be useful for the treatment of a variety of medical conditions, in which iron overload causes serious complications such as autoimmune and infectious arthritis.[27]

In view of growing antibiotic resistance, drug side-effects and resulting poor patient compliance, there is a need for new anti-microbial agents for *H. pylori*.

Our study confirmed that lactoferrin is not efficacious against *H. pylori* as a single agent.[13] Further studies are warranted to assess the previously described effect of lactoferrin as an additive to standard eradication therapies.[14]

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