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In Vitro Antimicrobial Activity and Downregulation of Virulence Gene Expression on Helicobacter pylori by Reuterin.

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Helicobacter pylori is an infectious agent commonly associated with gastrointestinal diseases. The use of probiotics to treat this infection has been documented, however, their potential antimicrobial metabolites have not yet been investigated. In the present study, the effect of reuterin produced by Lactobacillus reuteri on H. pylori growth and virulence gene expression was evaluated. It was observed that reuterin caused significant ($P < 0.05$) H. pylori growth inhibition at concentrations from 0.08 to 20.48 mM, with minimal inhibitory concentrations (MICs) of 20.48 mM for H. pylori ATCC700824 and 10.24 mM for H. pylori ATCC43504. In a reuterin bacterial killing assay, it was observed that half of the MIC value for H. pylori (ATCC700824) significantly ($P < 0.01$) reduced colony numbers from 5.65 ± 0.35 to 3.78 ± 0.35 Log_{10}CFU/mL after 12 h of treatment and then increased them to 5.25 ± 0.23 Log_{10} CFU/mL at 24 h; at its MIC value (20.48 mM), reuterin abrogated ($P < 0.01$) H. pylori (ATCC700824) growth after 20 h of culture. In addition, reuterin significantly ($P < 0.01$) reduced H. pylori(ATCC 43504) colony numbers from 5.65 ± 0.35 to 4.1 ± 0.12 Log10 CFU/mL from 12 to 24 h of treatment and abrogated its growth at its MIC value (10.24 mM), after 20 h of treatment. Reuterin did not alter normal human gastric Hs738.St/Int cell viability at the concentrations tested for H. pylori strains. Furthermore, 10 μM reuterin was shown to significantly ($P < 0.01$) reduce mRNA relative expression levels of H. pylorivirulence genes vacA and flaA at 3 h post-treatment, whose effect was higher at 6 h post-treatment, as measured by RT-qPCR. The observed direct antimicrobial effect and the downregulation of expression of virulence genes on H. pylori by reuterin may contribute to the understanding of the mechanisms of action of probiotics against H. pylori.

KEYWORDS:

Antimicrobial; Downregulation; Helicobacter pylori; Lactobacillus reuteri; Reuterin; Virulence gene

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Changes in gastric microbiota induced by Helicobacter pylori infection and preventive effects of Lactobacillus plantarum ZDY 2013 against such infection.

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Helicobacter pylori is a gram-negative pathogen linked to gastric ulcers and stomach cancer. Gastric microbiota might play an essential role in the pathogenesis of these stomach diseases. In this study, we investigated the preventive effect of a probiotic candidate Lactobacillus plantarum ZDY 2013 as a protective agent against the gastric mucosal inflammation and alteration of gastric microbiota induced by H. pyloriinfection in a mouse model. Prior to infection, mice were pretreated with or without 400 μL of L. plantarum ZDY 2013 at a concentration of 10(9) cfu/mL per mouse. At 6 wk postinfection, gastric mucosal immune response and alteration in gastric microbiota mice were examined by quantitative real-time PCR and high-throughput 16S rRNA gene amplicon sequencing, respectively. The results showed that L. plantarumZDY 2013 pretreatment prevented increase in inflammatory cytokines (e.g., IL-1β and IFN-γ) and inflammatory cell infiltration in gastric lamina propria induced by H. pylori infection. Weighted UniFrac principal coordinate analysis showed that L. plantarum ZDY 2013 pretreatment prevented the alteration in gastric microbiota post-H. pylori infection. Linear discriminant analysis coupled with effect size identified 22 bacterial taxa (e.g., Pasteurellaceae, Erysipelotrichaceae, Halomonadaceae, Helicobacteraceae, and Spirochaetaceae) that overgrew in the gastric microbiota of H. pylori-infected mice, and most of them belonged to the Proteobacteria phylum. Lactobacillus plantarum ZDY 2013 pretreatment prevented this alteration; only 6 taxa (e.g., Lachnospiraceae, Ruminococcaceae, and Clostridiaceae), mainly from the taxa of Firmicutes and Bacteroidetes, were dominant in the gastric microbiota of the L. plantarum ZDY 2013 pretreated mice. Administration of L. plantarum ZDY 2013 for 3 wk led to increase in several bacterial taxa (e.g., Rikenella, Staphylococcus, Bifidobacterium), although a nonsignificant alteration was found in the gastric microbiota. Overall, this study demonstrated that L. plantarum ZDY 2013 pretreatment played an important role in preventing gastric mucosal inflammation and gastric microbiota alteration induced by H. pyloriinfection, and the selective modulation in gastric microbiota posed by this intervention suggested that targeting gastric microbiota through oral administration of probiotics might be an alternative strategy to prevent H. pylori infection.

KEYWORDS:

Helicobacter pylori; Lactobacillus plantarum ZDY 2013; gastric microbiota; high-throughput; probiotic

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J Clin Gastroenterol. 2014 May-Jun;48(5):407-13. doi: 10.1097/MCG.0000000000000007.

Lactobacillus reuteri strain combination in Helicobacter pylori infection: a randomized, double-blind, placebo-controlled study.

Francavilla R[1], Polimeno L, Demichina A, Maurogiovanni G, Principi B, Scaccianoce G, Ierardi E, Russo F, Riezzo G, Di Leo A, Cavallo L, Francavilla A, Versalovic J.

GOALS:

The goals of this study were to investigate the role of a new probiotic preparation (Lactobacillus reuteri DSM 17938 and L. reuteriATCC PTA 6475) in Helicobacter pylori infection.

BACKGROUND:

Specific probiotic strains play a role in H. pylori infection for their ability to decrease bacterial load and gastritis, prevent antibiotic-associated side effects, and increase the eradication rate.

STUDY:

This is a prospective, double-blind, randomized, placebo-controlled study in a tertiary care setting. A total of 100 H. pylori-positive naive patients received either L. reuteri combination (2×10 Colony Forming Units) or placebo during a 3-phase study (pre-eradication, eradication, and follow-up). All underwent C urea breath test (C-UBT), blood assessments of gastrin-17 (G17), endoscopy, and the Gastrointestinal Symptom Rating Scale. Eradication was confirmed by C-UBT 8 weeks after the completion of therapy.

RESULTS:

Fifty patients were allocated in each group. During pre-eradication period, C-UBT δ decreased by 13% in L. reuteri combination as compared with a 4% increase in placebo (-13.2±34% vs. 4.3±27%; P<0.03). During eradication, GSRS increased significantly in placebo as compared with L. reuteri combination (6.8±2.9 vs. 4±3.1; P<0.01). Significantly less patients in L. reuteri combination as compared with placebo-reported side effects (40.9% vs. 62.8%; P<0.04). An abnormal G17 value was found in patients receiving placebo as compared with L. reuteri combination (28% vs. 12%; P<0.02). Eradication rate was 75% in L. reuteri combination and 65.9% in placebo (P=NS). L. reutericombination increased eradication rate by 9.1% (odds ratio: 1.5).

CONCLUSIONS:

L. reuteri combination alone is able to exert an inhibitory effect on H. pylori growth, and when administered with eradication therapy, it determines a significant reduction in antibiotic-associated side effects. Moreover, L. reuteri combination was able to decrease serum G17 levels and to (not significantly) increase the H. pylori-eradication rate.

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Helicobacter. 2014 Apr;19(2):144-55. doi: 10.1111/hel.12105. Epub 2014 Jan 5.

Anti-inflammatory properties of gastric-derived Lactobacillus plantarum XB7 in the context of Helicobacter pylori infection.

Thiraworawong T[1], Spinler JK, Werawatganon D, Klaikeaw N, Venable SF, Versalovic J, Tumwasorn S.

BACKGROUND:

Helicobacter pylori colonization of the gastric epithelium induces interleukin-8 (IL-8) production and inflammation leading to host cell damage. We searched for gastric-derived Lactobacillus with the ability to suppress H. pylori-induced inflammation.

MATERIALS AND METHODS:

Conditioned media from gastric-derived Lactobacillus spp. were tested for the ability to suppress H. pylori-induced IL-8 production in AGS gastric epithelial cells. IL-8 protein and mRNA levels were measured by ELISA and qPCR, respectively. The changes on host cell signaling pathway were analyzed by Western blotting and the anti-inflammatory effect was tested in a Sprague-Dawley rat model.

RESULTS:

Conditioned media from L. salivarius B101, L. rhamnosus B103, and L. plantarum XB7 suppressed IL-8 production and IL-8 mRNA expression in H. pylori-induced AGS cells without inhibiting H. pylori growth. Conditioned media from LS-B101, LR-B103, and LP-XB7 suppressed the activation of NF-κB in AGS cells, while strain LP-XB7 also suppressed c-Jun activation. The anti-inflammatory effect of LP-XB7 was further assessed in vivo using a H. pylori-infected Sprague-Dawley rat model. Strain LP-XB7 contributed to a delay in the detection and colonization of H. pylori in rat stomachs, attenuated gastric inflammation, and ameliorated gastric histopathology. Additionally, the administration of LP-XB7 correlated with the suppression of TNF-α and CINC-1 in sera, and suppression of CINC-1 in the gastric mucosa of H. pylori-infected rats.

CONCLUSIONS:

These results suggest that L. plantarum XB7 produces secreted factors capable of modulating inflammation during H. pyloriinfection, and this probiotic Lactobacillus strain shows promise as an adjunctive therapy for treating H. pylori-associated disease.

KEYWORDS:

Helicobacter pylori; Lactobacillus plantarum; Sprague-Dawley rat; interleukin-8; probiotic; stomach

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World J Microbiol Biotechnol. 2012 May;28(5):1871-80. doi: 10.1007/s11274-011-0984-z. Epub 2012 Feb 7.

Human lactoferrin increases Helicobacter pylori internalisation into AGS cells.

Coray DS[1], Heinemann JA, Tyrer PC, Keenan JI.

Helicobacter pylori has high global infection rates and can cause other undesirable clinical manifestations such as duodenal ulcer (DU) and gastric cancer (GC). Frequencies of re-infection after therapeutic clearance and rates of DU versus GC vary geographically and differ markedly between developed and developing countries, which suggests additional factors may be involved. The possibility that, in vivo, lactoferrin (Lf) may play a subtle role in modulating micronutrient availability or bacterial internalisation with implications for disease etiology is considered. Lf is an iron binding protein produced in mammals that has antimicrobial and immunomodulatory properties. Some bacteria that regularly colonise mammalian hosts have adapted to living in high Lf environments and we investigated if this included the gastric pathogen H. pylori. We found that H. pylori was able to use iron from fully iron-saturated human Lf (hLf) whereas partially iron-saturated hLf (apo) did not increase H. pylori growth. Instead, apo-hLf increased adherence to and internalisation of bacteria into cultured epithelial cells. By increasing internalisation, we speculate that apo-human lactoferrin may contribute to H. pylori's ability to persistence in the human stomach, an observation that potentially has implications for the risk of H. pylori-associated disease.

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J Food Sci. 2012 Jan;77(1):M9-14. doi: 10.1111/j.1750-3841.2011.02498.x. Epub 2011 Dec 19.

Antagonistic activities of lactobacilli against Helicobacter pylori growth and infection in human gastric epithelial cells.

Chen X[1], Liu XM, Tian F, Zhang Q, Zhang HP, Zhang H, Chen W.

Lactobacilli have positive effects on bowel microflora and health in humans and animals. In this study, the antagonistic activities of Lactobacillus gasseri Chen, and L. plantarum 18 were assessed by agar plate diffusion assay and tests that determined the growth and urease activity of Helicobacter pylori cocultured with lactobacilli and the adherence of H. pylori to human gastric epithelial cells in the presence of lactobacilli. The results showed that the 2 Lactobacillus strains had significant anti-H.pylori activity, and this activity may be contributed by the cell-free supernatants (CFS) of lactobacilli and live Lactobacillus strains in vitro. The antagonistic activity of the CFS against H. pylori depended on the pH and the presence of metabolites, such as organic acids and proteases. Our results also indicated that 2 Lactobacillus strains could inhibit H. pylori adherence human gastric epithelial cells.

PRACTICAL APPLICATION:

Helicobacter pylori causes chronic gastritis, peptic ulcer disease, and gastric cancer, and it infects about 50% of the world's population. Lactobacilli have been reported to have an inhibitory effect on H. pylori and can be used as probiotic to manufacture dairy products preventing H. pylori infection.

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Helicobacter. 2009 Apr;14(2):119-27. doi: 10.1111/j.1523-5378.2009.00666.x.

Meta-analysis: the effect of supplementation with lactoferrin on eradication rates and adverse events during Helicobacter pylori eradication therapy.

Zou J[1], Dong J, Yu XF.

Abstract

BACKGROUND:

Recent evidence shown that lactoferrin could exert an antimicrobial effect against Helicobacter pylori both in vitro and in vivo models. To systematically evaluate whether adding lactoferrin to H. pylori eradication regimens could improve eradication rates and reduce side-effects during anti-H. pylori treatment.

MATERIALS AND METHODS:

Eligible articles were identified by searches of electronic databases. We included all randomized trials comparing lactoferrin supplementation to placebo or no treatment during anti-H. pylori regimens. Statistical analysis was performed with Review Manager 5.0.10. Subanalysis/Sensitivity analysis was also performed.

RESULTS:

We identified nine randomized trials (n = 1343). Pooled H. pylori eradication rates were 86.57% (95% confidence interval (CI) = 83.99-89.15%) and 74.44% (95% CI = 71.14-77.74%) for patients with or without lactoferrin by intention-to-treat analysis, respectively, the odds ratio (OR) was 2.26 (95% CI = 1.70-3.00); the occurrence of total side-effects was 9.05% (95% CI = 6.83-11.27%) and 16.28% (95% CI = 13.43%-19.13%) for groups with or without lactoferrin, especially for nausea, the summary OR was 0.15 (95% CI = 0.04-0.54).

CONCLUSIONS:

Our review suggests that supplementation with lactoferrin could be effective in increasing eradication rates of anti-H. pyloritherapy, and could be considered helpful for patients with

eradication failure. Furthermore, lactoferrin shows a positive impact on H. pyloritherapy-related side-effects.

Aliment Pharmacol Ther. 2009 Apr 1;29(7):720-30. doi: 10.1111/j.1365-2036.2009.03934.x.

Meta-analysis: efficacy of Bovine lactoferrin in Helicobacter pylori eradication.

Sachdeva A[1], Nagpal J.

Abstract

BACKGROUND:

Several randomized-controlled trials (RCTs) have sought to determine the efficacy of bovine lactoferrin in Helicobacter pylorieradication with equivocal results.

AIM:

To evaluate the effect of bovine lactoferrin supplementation in H. pylori eradication.

METHODS:

Electronic databases, reviews, bibliographies, abstracts and conference proceedings were searched. Included trials had to be randomized or quasi-randomized and controlled, using bovine lactoferrin in the intervention group, treating Helicobacter-infected subjects and evaluating eradication of H. pylori as an outcome.

RESULTS:

The search identified five eligible RCTs (of 169). Data were available for 682 subjects (bovine lactoferrin group-n = 316; control group-n = 366). The pooled odds ratio (five studies) for eradication by intention-to-treat analysis was 2.22 (95% CI 1.44-3.44; P = 0.0003) using the fixed effects model (FEM) and 2.24 (95% CI 1.15-4.35; P = 0.0003) using the random effects model (REM) (Cochran's Q = 6.83; P = 0.145). The pooled risk difference was 0.11 (95% CI 0.05-0.16; P = 0.0001) by FEM (Cochran's Q = 6.67; P = 0.154) and 0.10 (95% CI 0.04-0.17; P = 0.0023) by REM. There was no significant difference in incidence of adverse effects.

CONCLUSION:

Bovine lactoferrin potentially improves H. pylori eradication rates without any impact on adverse effects, but available evidence is limited and further research is necessary to confirm the findings.

Am J Gastroenterol. 2007 May;102(5):951-6. Epub 2007 Feb 21.

Helicobacter pylori eradication: a randomized prospective study of triple therapy versus tripletherapy plus lactoferrin and probiotics.

de Bortoli N[1], Leonardi G, Ciancia E, Merlo A, Bellini M, Costa F, Mumolo MG, Ricchiuti A, Cristiani F, Santi S, Rossi M, Marchi S.

Abstract

OBJECTIVES:

Helicobacter pylori is causally associated with gastritis and peptic ulcer diseases. Recent data (meta-analysis) have demonstrated that triple therapy with amoxicillin, clarithromycin, and a proton pump inhibitor has an eradication rate of only 74-76% and new therapeutic protocols may be necessary. The aim of this study was to examine whether adding bovine lactoferrin (bLf) and probiotics (Pbs) to the standard triple therapy for H. pylori infection could improve the eradication rate and reduce side effects.

METHODS:

H. pylori infection was diagnosed in 206 patients: in 107 based on an upper endoscopy exam and a rapid urease test, and in 99 by means of the H. pylori stool antigen-test and the C(13) urea breath test (C(13) UBT). The patients were randomized into two groups: 101 patients (group A) underwent standard triple eradication therapy (esomeprazole, clarithromycin, amoxicillin), while 105 patients (group B) underwent a modified eradication therapy (standard triple eradication therapy plus bLf and Pb). Successful eradication therapy was defined as a negative C(13) UBT 8 wk after completion of the treatment. Results were evaluated by intention-to-treat (ITT) and per-protocol (PP) analysis. Data were evaluated and considered positive when $P<0.05$.

RESULTS:

At the end of the study 175/206 patients showed negative C(13) UBT results. According to intention-to-treat analysis, the infection was eradicated in 73/101 patients from Group A and in 93/105 from Group B. PP analysis showed 73/96 patients from Group A and 93/101 from Group B to have been successfully treated. More patients from group A than from group B reported side effects from their treatment ($P<0.05$).

CONCLUSIONS:

The results of our study suggest that the addition of bLf and Pbs could improve the standard eradication therapy for H. pyloriinfection--bLf serving to increase the eradication rate and Pbs to reduce the side effects of antibiotic therapy.