Effect of proton pump inhibitor on the movement of *Helicobacter pylori* across the mucus layer to the gastric lumen

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) cannot usually be detected in the gastric juice and it is thought that *H. pylori* may reside under the mucus layer for long term. The mechanisms of action of proton pump inhibitor (PPI) for *H. pylori* eradication are not entirely clear. Our study aimed to determine the role of PPI on the movement of *H. pylori* across the mucus layer to the gastric lumen and the mechanism of PPI on *H. pylori* eradication. **Methods:** Patients with *H. pylori* infection were intravenously injected with PPI (intervention group, n=31) or without PPI (control group, n=37). The presence of *H. pylori* in the gastric juice was evaluated by the rapid urease test (RUT), polymerase chain reaction (PCR), and culture methods. **Results:** The *H. pylori* positive detection rates were all significantly higher among patients in the intervention group than among patients in the control group by the RUT ($P < 0.0001$), PCR ($P < 0.0001$), and culturing ($P = 0.0386$). **Conclusion:** *H. pylori* can penetrate across the mucus layer to the gastric lumen following PPI intervention, and thus it might represent a novel target in the eradication of *H. pylori*.

Background

Helicobacter pylori (*H. pylori*) is one of the most common bacterial infections, potentially lasting for decades in an individual, and infection can lead to multiple diseases. The prevalence of *H. pylori* infection is higher than 50% in many regions of the world, for example 68.6% in Chile, 71.6% in Italy¹, 51.7% in Japan, 63.5% in India, and 35.6% in United States². It ranges from 41.35% to 72.3% in China, with an average of 56.22%.³ *H. pylori* is widely regarded as one of the most common gastric pathogens⁴⁻⁶ causing chronic gastritis, functional dyspepsia, peptic ulcer, gastric adenocarcinoma, and lymphoma.⁷ It has also been found to be associated with multiple extra gastrointestinal diseases, such as cardiovascular diseases, hematological system diseases, diabetes, and immune diseases. In 1994, the International Agency for Research on Cancer consensus group listed *H. pylori* as a class I human carcinogen.⁹ The eradication of *H. pylori* is a major global public health issue. Currently, several diagnostic tests are available for determining the presence of *H. pylori*, such as rapid urease test (RUT), histology, polymerase chain reaction (PCR), culture, urea breath test (UBT), and serology.

At present, the standard method for the eradication of *H. pylori* is triple (proton pump inhibitor [PPI] + two kinds of antibiotics) or quadruple (PPI + two kinds of antibiotics + bismuth) therapy based on PPI recommended by the international guidelines from the Maastricht V consensus,¹⁰ the Toronto consensus,¹¹ and other consensuses from many countries.¹²⁻¹⁵ However, using this traditional treatment, it has become increasingly difficult to eradicate *H. pylori* because of the side effects and increasing antibiotic resistance.^{16, 17} The search for alternative treatments, such as microecological agents, traditional Chinese medicine, second-line antibiotics (such as Rifabutin), and vaccines¹⁸ has become a particular focus. However, *H. pylori* eradication remains a serious challenge. Until now, the mechanism of action of PPI, antibiotics, and bismuth for *H. pylori* eradication has not been fully understood. A new

understanding of the *H. pylori* eradication mechanism is needed to tailor accurate and effective *H. pylori* eradication therapy.

H. pylori can invade into the surface of the gastric mucosa and penetrate across the mucin layer. We therefore hypothesized that *H. pylori* may also penetrate across the mucin layer to the gastric lumen. In this study, we investigated the movement of *H. pylori* up and down the gastric mucus layer and analyzed the effect of PPI on the movement of *H. pylori*.

Methods

Ethical considerations

The Ethics Committee of Central Hospital of Cangzhou City, Cangzhou, Hebei, China, approved this study, which was performed in accordance with the ethical guidelines of the Declaration of Helsinki, Good Laboratory Practices and Good Clinical Practices (2018-017-01). Written informed consent was obtained from each patient prior to study enrolment.

Patients and specimens

A total of 80 patients infected with *H. pylori* were recruited from Central Hospital of Cangzhou City from March 2018 to May 2018. *H. pylori* infection was diagnosed on the basis of the RUT results following gastric biopsy or a positive UBT result. Eligible patients were ≥ 18 years of age. Subjects meeting with the following criteria were excluded: co-morbidities with severe cardiovascular disease, liver disease or other infectious disease, had received PPI or been administered histamine-2 receptor blocker (H2RB), antibiotics or other treatments that may affect *H. pylori* detection in the previous week, or had a history of gastric surgery. Patients were divided into two groups randomly: (1) Control group: patients with no previous treatment for *H. pylori* eradication, (2) Intervention group: patients who received PPI, prevacid, intravenous infusion 1–2 h before undergoing gastrointestinal endoscopy examination. Patients in each group did not receive any other drugs. Esophagogastroduodenoscopy was performed after an 8 h fast without any defrother, anesthetic or other orally-administered drugs. Patients lay on the left recumbent position of the examining table and were successfully anesthetized by intravenous injection of propofol. Then 2–3 ml of fasting gastric juice not containing blood, bile or any other components were sampled from the fundus/corpus at endoscopy by means of a sterile cannula with an external connection to a 10 ml sterile syringe. The pH of the gastric juice was determined and a Giemsa stain was performed. The gastric fluid was dropped into a RUT reagent bottle (SanQiang Biological and Chemical Co. Ltd., Fujian, China) and was observed for at least 30 min at room temperature. After each examination, the endoscope was washed with 2% glutaraldehyde and disinfected with 70% ethanol followed by rinsing with sterile water. The sterile cannula was reformed from a sprinkler tube by cutting off its front end, which was then washed with sterile water, treated with an enzymatic hydrolysate, and doused in glutaraldehyde for at least 10 h, followed by rinsing with sterile water after each examination.

Bacterial cultivation

Culturing of the gastric fluid samples to detect *H. pylori* was performed using the *Helicobacter pylori* isolated, Verification and Antibiotics Susceptibility Testing Kit (Zhuhai Special Economic Zone Yimin Biological Engineering Products Factory, Zhuhai, China). The gastric fluid was inoculated onto Brucella broth supplemented with equine serum and antibiotics (vancomycin, bacillosporin, amphotericin B, and trimethoprim). Culture plates were incubated under microaerophilic conditions at 37°C and high humidity for 1–2 days. The change in color and turbidity of the culture liquid was observed, with a turbid, red culture liquid suggesting *H. pylori* growth (Fig. 1). Organisms were identified as *H. pylori* based on both positive catalase (Fig. 2) and urease tests (Fig. 3).

PCR analysis of *H. pylori* DNA from gastric juice

H. pylori DNA was isolated and analyzed by PCR using Diagnostic Kit of *Helicobacter pylori* DNA (PCR-Fluorescence Probing) (Daangene, Guangzhou, China) according to the manufacturer's recommendations. Take 50µl gastric juice into a 1.5ml centrifuge tube, add 50u1 purified water and 50µl DNA extract reagent into the centrifuge tube and mix it thoroughly, and then centrifuge it at 12,000 RPM for 5 minutes. Specific primers and fluorescent probes for the highly conserved coding region of *H. pylori* genome as the target region were included in this kit. The reaction was performed using the ABI 7500 detection system (Applied Biosystems, Foster, CA, USA) with preliminary denaturation for 8 min at 50°C, 2 min at 93°C, followed by 10 amplification cycles of denaturation at 93°C for 45 s and annealing at 55°C for 1 min, followed by 30 amplification cycles of denaturation at 93°C for 30 s, annealing at 55°C for 45 s, and primer extension at 55°C for 45 s, with a fluorescence acquisition step at the end of the extension. The reference value for this kit was 27.02, which was determined by the ROC curve method. Samples were considered to be *H. pylori* positive when the amplification curve was of typical 'S' type and the Ct value was ≤ 27.02 .

Statistical analysis

Calculations were performed using SPSS software version 22(IBM Corp., Armonk, NY, USA). Descriptive analysis was performed for demographic and clinical features. Results are presented as the mean $\pm$ SD for quantitative variables and the number (percentage) for qualitative variables. The Chi-square test was used to compare categorical data. The *T*-test was used to compare normally distributed continuous variables. $P < 0.05$ indicated a statistically significant difference.

Results

Patients' characteristics

Table 1 presents the patients' characteristics. The gastric fluid was collected from 68 patients infected with *H. pylori* after the gastric fluid mixed with blood or bile excluded. The pH value of the gastric juice was significantly higher in the intervention group than in the control group ($P < 0.0001$).

Comparison of gastric fluid by the culture method

None of the gastric fluid cultures were positive for *H. pylori* in the control group (0%), whereas four cultures positive for *H. pylori* were detected in the intervention group (12.9%). The positive frequency was higher in the intervention group than in the control group ($P = 0.0386$).

Comparison of gastric fluid by the RUT method

Among the 37 patients in the control group, the gastric fluid sample from one patient gave a positive RUT result (2.7%) compared with 14 positive cases among the 31 patients in the intervention group (45.2%). The positive frequency was higher in the intervention group than in the control group ($P < 0.0001$).

Comparison of gastric fluid by the PCR method

PCR of the gastric fluid revealed six positive samples among the control group (16.2%) compared with 20 positive samples among the intervention group (64.5%). The positive frequency was higher in the intervention group than in the control group ($P < 0.0001$) (Fig. 4).

Discussion

H. pylori is a Gram-negative microaerobic bacterium that is spiral in shape and has 2–6 polar flagella for mobility. This bacterium resides between the surface of the gastric mucosa and the mucin layer without evidence of intracellular parasitism.¹⁹ *H. pylori* can only survive at a periplasmic pH of 4.0–8.5 and can only grow at a periplasmic pH of 6.0–8.5. It is difficult to detect *H. pylori* in the gastric lumen because it is such an acidic environment.

The human stomach is divided into three anatomic regions: the cardia, the fundus/corpus, and the antrum. The antrum secretes alkaline mucus 4–5 cm around the antrum. Therefore, *H. pylori* is mainly distributed in the antrum.²⁰ *H. pylori* distribute in two ways: (1) by colonizing the surface of the gastric pit and epithelial cells, and (2) by colonizing above the tissue surface mucus layer. The latter is more common. An animal model revealed that *H. pylori* colonizes a zone 0–25 μm above the tissue surface mucus layer, to a total thickness of about 100 μm .²¹ While the gastric lumen has a pH of 1–2, a pH gradient exists across the mucus layer, reaching a pH of 6–7 at the surface of the mucosa.²² Using chemotaxis, *H. pylori* navigate this pH gradient to reach their niche environment near the host epithelium.^{23,24}

Research has revealed that the stomach supports a bacterial community comprising hundreds of phylotypes,^{25, 27} while a pH of < 4 prevents bacterial overgrowth. It was reported that the microbial density in the stomach is 10^1 – 10^3 CFU/g^{28, 29} This high density of bacteria means that Giemsa staining is not an optimal method for studying *H. pylori* in the stomach. Therefore, in this study, we employed the RUT, PCR analysis, and culture methods to study *H. pylori* in gastric juice.

Until now, little is known about the movement characteristics of *H. pylori* all over the world. We use PPI intervene the patients infected with *H. pylori* and study the movement characteristics of *H. pylori*. In this

study, the pH of gastric juice in control group patients was range from 1 to 3 with an average of 1.59, which was consistent with normal pH value of gastric juice. The pH of the gastric juice of patients in the intervention group was higher than that in the control group, but *H. pylori* was detected in both groups. Culture is the gold standard method for detecting the presence of viable *H. pylori*. In our study, the positive detection rate was significantly higher in the gastric juice of patients in the intervention group than of patients in the control group. The RUT and PCR analysis also indicated that positive detection rates were significantly higher among patients in the intervention group than among those in the control group. The results of culturing, the RUT, and PCR were consistent. Our findings indicated that PPI intervention may induce *H. pylori* to penetrate across the mucus layer from the surface of the gastric mucosa to the gastric lumen. However, one positive case was detected by the RUT of the gastric fluid from the control group and this sample had a pH value of 1, possibly indicating that the gastric fluid may be mixed with slight bile. In addition, six samples from the control group tested positive by PCR, which might indicate that there was a high load of *H. pylori* for these bacteria to be detected in the gastric lumen.

H. pylori is a fastidious microorganism that requires complex growth media. The *H. pylori* living environment is small in the mucus layer and a key feature of this bacterium is its microaerophilicity, with optimal growth at O₂ levels of 2% to 5%.³⁰ *H. pylori* is unable to survival under normal atmospheric conditions or under absolute anaerobic conditions. *H. pylori* requires complex growth media rich in nutrients. However, the mucus layer has low permeability to most molecules such as protons, O₂, and nutrient macromolecules. If the condition of juice in gastric lumen met the qualification mentioned above, *H. pylori* may penetrate across the mucus layer to the gastric lumen.

It is reported that PPI alone also exerts direct antimicrobial activity against *H. pylori* with 6%–7.7% eradication rate,^{31,32} which was confirmed by Meining and colleagues.³³ Iwahi and coworkers³⁴ practiced a more in-depth study of PPI, and surprisingly found that lansoprazole could inhibit the growth of *H. pylori in vitro*, similar to the antibacterial effects of antibiotics. A series of subsequent studies found that omeprazole, pantoprazole, and rabeprazole have different degrees of inhibitory effects on *H. pylori in vitro*, with rabeprazole showing the strongest bacteriostatic effect. However, these differing effects of PPIs have not been reported in clinic. According to our research results, we propose that PPI intervention may reduce the pH gradient, disturbing the pH chemotaxis of *H. pylori* and leading to the penetration of the mucus layer. The flagella of *H. pylori* often carry a distinctive bulb at the end. The flagella confer motility and allow rapid movement in viscous solutions such as the mucus layer overlying the gastric epithelial cells.³⁵ Motile bacteria sense chemical gradients by means of chemoreceptor proteins, such as BabA, with a pH–sensor mechanism³⁶, and relay the information to the flagellar motor³⁷ to direct movement toward an environment with optimal concentrations of both electron acceptors and proton/electron donors. Another study reported that among *H. pylori* exposed to neutral pH, only about 7% of the culture were motile and traveled at an average speed of 10.5 µm per s. By contrast, among cells that were shifted to an acidic pH, 66% were motile with a significantly faster average speed of 24.3 µm per s³⁸. It seems likely that *H. pylori* uses the pH-sensing mechanism for orientation along the

transmucus pH gradient. *H. pylori* can penetrate across the mucus layer to the gastric lumen when the pH gradient is reduced by PPI intervention. By the results in this study, *H. pylori* can be more easily detected in whom received intravenous PPI infusion which may imply the *H. pylori* in gastric lumen following PPI intervention. However, there are several factors involving the motility of *H. pylori* further investigation may be necessary

Conclusions

Our study indicated that *H. pylori* can penetrate the mucus layer and enter the gastric lumen where it acquires O₂ and nutrients under PPI intervention. The mechanism of direct antimicrobial activity of PPI may be that PPI disturbs the pH chemotaxis of *H. pylori*, leading to penetration across the mucus layer, where it is diminished by probiotics above the mucus layer and eliminated by gastric emptying. Through the movement characteristic of *H. pylori*, we provide reliable data for a theoretical basis to improve the eradication of *H. pylori*.

Abbreviations

H. pylori: *Helicobacter pylori*; PPI: proton pump inhibitor; RUT: rapid urease test; PCR: polymerase chain reaction; UBT: urea breath test

Declarations

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Authors' contributions

ZGQ designed the study and the study protocol, and modified the paper. SSS and LYY performed the experiments and analyzed the data. SS wrote the paper. LYF and SH performed gastrointestinal endoscopy examination. YWJ and ZGZ collected the cases.

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analysis and interpretation of data and in writing the manuscript.

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Ethics Committee of Central Hospital of Cangzhou City, Cangzhou, Hebei, China, approved this study, which was performed in accordance with the ethical guidelines of the Declaration of Helsinki, Good Laboratory Practices and Good Clinical Practices (2018-017-01). Written informed consent was obtained from each patient prior to study enrolment.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interest.

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Table 1

Table 1 Characteristics of the patients

Parameter	Control group (n=37)	Intervention group (n=31)	P-value
Gender (M/F)	20/27	19/12	0.548
Age, mean±SD	53.2±11.7	55.8±12.8	0.256
pH value of gastric juice, mean±SD	1.59±0.76	6.70±1.42	<0.001

Figures

(A)

(B)

Figure 1

Culture liquid turbidity and red suggesting *H. pylori* growth. (A) Negative result. (B) Positive result.

(A)

(B)

Figure 2

H. pylori can generate catalase which decompose H_2O_2 and released bubbles in micropore labeled 'CAT'. Bubbles can be seen in CAT micropore was identified as *H. pylori* positive. (A) Negative result. (B) Positive result.

(A)

(B)

Figure 3

H. pylori can generate urease which decompose the urea, released ammonia in micropore labeled 'Ur+', and the ammonia can turn the phenol red from yellow to red colour. The Ur+ micropore turned from yellow to red colour was identified as *H. pylori* positive. (A) Negative result. (B) Positive result.

Control group

Intervention group

Figure 4

Comparison of gastric fluid PCR for *H. pylori* with and without PPI. The positive frequency was higher in PPI intervention group than that in Control group ($P < 0.001$).

Figure 5

The movement characteristics of *H. pylori* under PPI intervention and the mechanisms of action of PPI, antibiotics and probiotics.