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ORIGINAL ARTICLE

# Celecoxib inhibits *Helicobacter pylori* colonization-related factors

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## Abstract

**AIM:** To investigate the effect of celecoxib, a selective COX-2 inhibitor, on *Helicobacter pylori* (*H. pylori*) colonization-related factors and its mechanism.

**METHODS:** After co-incubation with celecoxib, morphology of *H. pylori* strain 26695 was observed under a transmission electron microscope. Flagella motility was assessed by stab agar motility test. Adherence of *H. pylori* to AGS cells was determined by enzyme linked immunosorbent assay. Levels of mRNA expression in flagellar genes (*flaA*, *flaB*), urease genes (*ureA*, *ureB*) and adhesin genes (*babA*, *sabA*, *alpA*, *alpB*, *hpaA*, *hopZ*) were measured by real-time polymerase chain reaction.

**RESULTS:** Separation and non-integrity of bacterial cell wall, rarefaction and asymmetry of cytoplasm, and even lysis of *H. pylori* were observed in the presence of celecoxib. When *H. pylori* strains were incubated in the presence of celecoxib, their flagellar motility and adherence to AGS cells were inhibited. The expression of *ureA*, *ureB*, *babA*, *sabA*, *alpA*, *alpB*, *hpaA*, *hopZ* was up-regulated while the expression of *flaA*, *flaB* was down-regulated in the presence of celecoxib.

**CONCLUSION:** Celecoxib inhibits flagellar motility and adherence of *H. pylori* to AGS cells, and destructs their normal structure *in vitro*.

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**Key words:** *Helicobacter pylori*; Celecoxib; Colonization; Ultrastructure

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## INTRODUCTION

About 30% of the population in developed countries and up to 90% of the population in developing countries are chronically infected with *Helicobacter pylori* (*H. pylori*)[1,2]. Non-steroidal anti-inflammatory drugs (NSAID) are the most commonly used drugs, on a world-wide scale, which are used by at least 30 million people[3]. NSAID and *H. pylori* infection are two major factors for gastric injuries. Subjects taking NSAID are often infected with *H. pylori*. However, whether these two factors exert synergistic or antagonistic actions on gastric mucosa is still controversial[4,5]. Data from a meta-analysis review have shown that the risk of peptic

ulcer is approximately 60-fold higher in *H. pylori* positive subjects taking NSAID than in *H. pylori* negative subjects not taking NSAID[6]. Since both *H. pylori* and NSAID are responsible for mucosal damage, they can increase the risk of developing uncomplicated and complicated peptic ulcer. However, data from several studies do not always confirm such an assumption[5]. A large clinical trial demonstrated that eradication of *H. pylori* delays the healing of gastric ulcers in NSAID users after treatment with omeprazole[7], implying that *H. pylori* may protect individuals against NSAID-induced ulcer, possibly by stimulating mucosal prostaglandins and other protective factors.

Recent studies *in vitro* also suggested that aspirin and celecoxib, a selective COX-2 inhibitor, inhibit the growth of *H. pylori* and decrease the activity of urease and vacuolating cytotoxin in a dose-dependent manner[8-12], indicating that NSAID may antagonize injuries of gastric mucosa caused by *H. pylori* infection. Colonization of *H. pylori* in gastric mucosa is a prerequisite for pathogenicity and needs to have at least 4 basic characteristics: integrate helical shape, motility of flagella, specific binding to adhesin and its receptors, and urease activity that provides an appropriate microenvironment[13]. We hypothesize that NSAID and celecoxib may influence the pathogenicity of *H. pylori* in gastric mucosa injury by altering the colonization. Therefore, the aim of the present study was to investigate the effect of celecoxib on *H. pylori* colonization- related factors and its mechanism *in vitro*.

## MATERIALS AND METHODS

### Bacterial culture

*H. pylori* 26695 strain was cultured at 37℃ in a microaerobic atmosphere containing 5% $O_2$, 85% $N_2$, and 10% $CO_2$ for 48 h on Colombia agar medium supplemented with 8% (v/v) defibrinated goat blood containing 0.02 mmol/L celecoxib or vehicle control (1/1000 DMSO).

### Stab agar motility test

*H. pylori* strains were grown on Colombia agar medium for 48 h and then harvested into a brain heart infusion (37 g/L). After the concentration of bacteria was adjusted to $10^8$ CFU/mL, 10 μL was inoculated into a 0.3% agar Brucella broth medium containing 8% defibrinated goat blood using a sterile picker. Five days after incubation under microaerobic condition at 37℃, the halo diameter was measured.

### Ultrastructural analysis

Forty-eight hours after exposure to 0.02 mmol/L celecoxib, *H. pylori* cells were collected and rinsed three times with 0.01 mol/L PBS, fixed in phosphate-buffer solution containing 2.5% glutaraldehyde at 4℃ for 2 h. After centrifugation, pellets were embedded in 2% agar, fixed in 1% osmium tetraoxide (OsO4) at 4℃, and rinsed three times with 0.01 mol/L PBS. After dehydrated in a series of graded acetone at 4℃, specimens were embedded in Epon 812 (Emicron). The sample was cut into 90 nm-thick sections which were stained with uranyl acetate and lead citrate, and observed under a JEM1230 transmission electron microscope.

### Adhesion of H. pylori to AGS cells

AGS cells ($10^4$/well) were seeded in RPMI 1640 medium (Gibco) containing 10% fetal bovine serum in a 96-well plate containing 5% $CO_2$ at 37℃ for 20 h. *H. pylori* ($10^7$ CFU/well) were pretreated with celecoxib at the concentrations of 0.01, 0.02 and 0.03 mmol/L. The plate was agitated at 60 r/min for 30 min at 37℃. Cultures were fixed with 1% paraformaldehyde. After washed with PBS, *H. pylori* cells were blocked with 5% bovine serum albumin (BSA) for 30 min, and incubated for 24 h with mouse monoclonal anti-*H. pylori* antibody (Santa cruz). After washed three times with PBS, goat anti-mouse IgG-HRP (Santa cruz) was added for 1 h. Binding was visualized by incubating with 100 μL TMB substrate for 30 min. Absorbance was read at 450 nm after 2 mol/L of sulphuric acid was added to terminate the reaction. Adherence of *H. pylori* to AGS cells was calculated according to the formula: [(*A* AGS cells with *H. pylori* -*A* AGS cells without *H. pylori*)/(*A* positive control -*A* negative control)] × 100. For positive control, only bacteria were added and allowed to adhere to the well. Wells containing neither AGS cells nor *H. pylori* were prepared as a negative control.

### H. pylori RNA isolation and reverse transcription

Forty-eight hours after pretreatment with 0.02 mmol/L celecoxib, strains of *H. pylori* were rinsed with Tris-HCl and cleared with 1 mL of TRIzol. After 200 μL of chloroform was added, the sample was vigorously shaken and centrifuged. RNA in aqueous phase was precipitated with 0.5 mL of isopropanol. The pellet was washed with ethanol and dried. The RNA was resuspended in sterile water and quantified by UV absorbance. Total RNA (4 μg) treated with RO1 RNase-free DNase (Promega) to remove DNA was used for reverse transcription reaction. In brief, 1.5 μL of random primers was added, the samples were heated to 70℃ for 5 min. Then, 10 μL of 5 × RT buffer, 2.5 μL of dNTPs, and 2 μL of M-MLV were added. cDNA synthesis reaction was performed for 60 min at 37℃ and then at 70℃ for 10 min. Aliquots of cDNA were stored at -70℃.

### Real-time polymerase chain reaction (RT-PCR)

mRNA levels of flagellar genes (*flaA, flaB*), urease genes (*ureA, ureB*) and adhesin genes (*babA, sabA, alpA, alpB, hpaA, hopZ*) were measured by real-time PCR using the ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems, Foster City, Calif). Specific primers and house-keeping gene 16SrRNA were designed with the aid of Primer Express 3.0 software (Applied Biosystem Perkin-Elmer) (Table 1). Real-time PCR was performed in a 25 μL reaction volume containing 2.5 μL

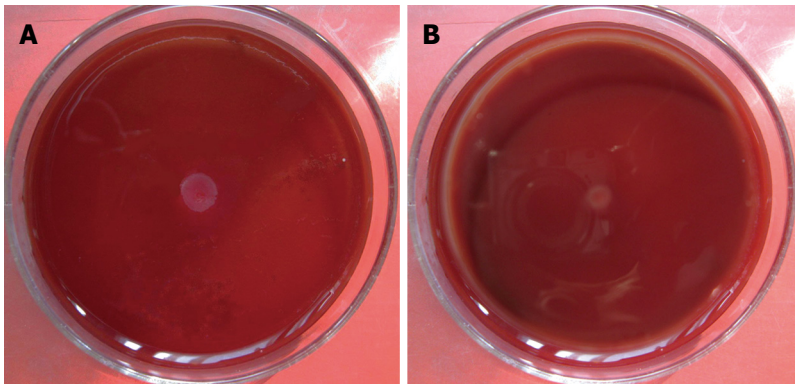


**Figure 1 Stab agar motility tests showing the *H. pylori* motility.** A: DMSO control (1/1000); B: Celecoxib (0.02 mmol/L).

**Table 1 Primers and probes used in real-time quantitative PCR**

| Gene | Primer (5′-3′) |
|---|---|
| *flaA-F* | ATTGGCGTGTTAGCAGAAGTGA |
| *flaA-R* | TGACTGGACCGCCACATC |
| *flaB-F* | ACATCATTGTGAGCGGTGTGA |
| *flaB-R* | GCCCCTAACCGCTCTCAAAT |
| *ureA-F* | GCTGGTGCGATTGGCTTTA |
| *ureA-R* | GGATAGCGACTTGCACATCGT |
| *ureB-F* | TCCTGATGGGACAAAACTCGTA |
| *ureB-R* | ACGGCTTTTTTGCCTTCGT |
| *babA-F* | TGCTCAGGGCAAGGGAATAA |
| *babA-R* | ATCGTGGTGGTTACGCTTTTG |
| *sabA-F* | GGTGTGCTGCAACAGACTCAA |
| *sabA-R* | CATAAGCTGTTGCGCCAAATT |
| *alpA-F* | GCACGATCGGTAGCCAGACT |
| *alpA-R* | ACACATTCCCCGCATTCAAG |
| *alpB-F* | ACGCTAAGAAACAGCCCTCAAC |
| *alpB-R* | TCATGCGTAACCCCACATCA |
| *hpaA-F* | GAGCGTGGTGGCTTTGTTAGT |
| *hpaA-R* | TCGCTAGCTGGATGGTAATTCA |
| *hopZ -F* | GCGCCGTTACTAGCATGATCA |
| *hopZ-R* | GAAATCTTTCGGCGCGTTT |
| *16SrRNA-F* | CCGCCTACGCGCTCTTTAC |
| *16SrRNA-R* | CTAACGAATAAGCACCGGCTAAC |

PCR: Polymerase chain reaction.

of cDNA, 12.5 μL of SYBR green real time PCR master mix (Toyobo), 1 μL of sense and antisense primers (5 pmol/L), and 9 μL of DEPC water. PCR was carried out at 95℃ for 10 min, followed by 40 cycles at 95℃ for 15 s, at 61℃ for 1 min. A further melting curve step analyzing the purity of PCR products was performed at 95℃ for 15 s, at 61℃ for 30 s, and at 96℃ for 15 s. A standard curve was plotted using 10-fold serial dilution of each cDNA. mRNA level was expressed as the ratio of detected mRNA to 16S rRNA mRNA [detected mRNA (U/mL)/16S rRNA mRNA (U/mL) × 100000]. PCR was carried out in quintuple using samples prepared at the same time.

### Statistical analysis

All experiments were performed at least in triplicate. Data were presented as mean ± SD. Statistical analysis between sample and control was conducted by Student's *t*-test using SPSS 11.0 software. $P < 0.05$ was considered statistically significant.

## RESULTS

### Effects of celecoxib on H. pylori motility

The halo diameter for the growth of *H. pylori* in the presence of celecoxib was 5.92 ± 1.20 mm after 5-d incubation, which was significantly smaller than that (8.21 ± 1.63 mm) of DMSO control ($P < 0.05$, Figure 1), indicating that the motility of *H. pylori* is decreased in the presence of celecoxib.

### Ultrastructural effects of celecoxib on H. pylori

Transmission electron microscopy demonstrated that both cytoplasmic and outer membranes of *H. pylori* were intact, the cytoplasm was well-distributed and the electron density was moderate in DMSO control. When incubated with 0.02 mmol/L of celecoxib, V- and U-shaped *H. pylori* were observed. The cell wall of *H. pylori* was attenuated with abscission, or even perforation but no integrity. Separation of the outer membrane from the cytoplasmic membrane (cell wall breakaway) and even cell lysis were observed. Rarefaction and asymmetry were observed in cytoplasm of *H. pylori* and the components of *H. pylori* cells disappeared and distributed abnormally (Figure 2).

### Effects of celecoxib on H. pylori adherence to AGS cells

Compared to the DMSO control (1/1000), celecoxib significantly inhibited the adherence of *H. pylori* to AGS cells in a dose-dependent manner ($P < 0.05$) (Figure 3).

### Effects of celecoxib on H. pylori flagellin, urease and adhesin gene expression

The mRNA expression levels in flagellar genes (*flaA, flaB*), urease genes (*ureA, ureB*) and adhesin genes (*babA, sabA, alpA, alpB, hpaA, hopZ*) were measured by real-time PCR. After treatment with 0.02 mmol/L celecoxib, the mRNA expression levels in *flaA* and *flaB* were lower than those in DMSO control ($P < 0.05$). However, the mRNA expression levels were higher in urease genes (*ureA, ureB*) and adhesin genes (*babA, sabA, alpA, alpB, hpaA, hopZ*) than in DMSO control ($P < 0.05$). The

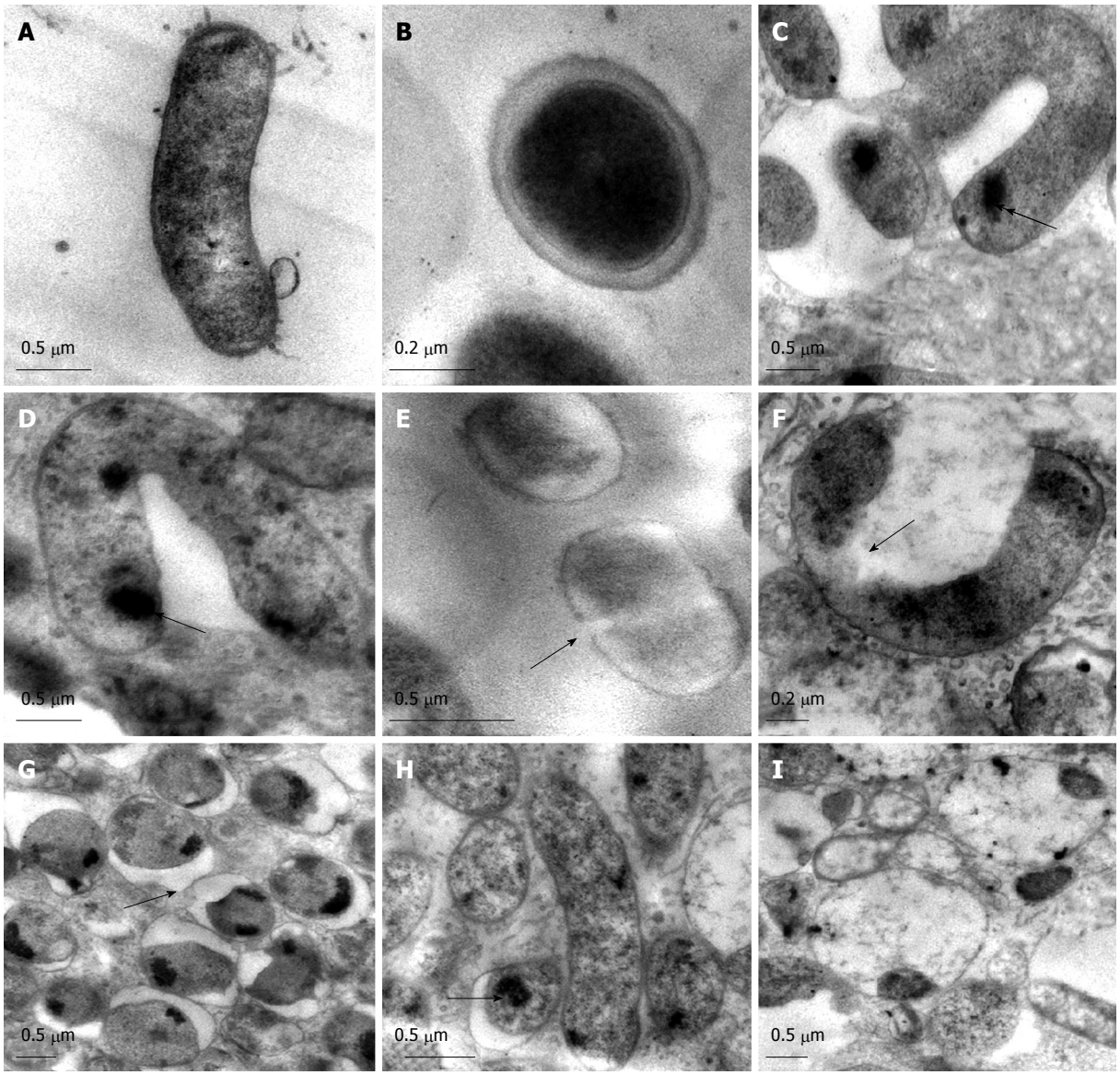


**Figure 2 Transmission electron microscopy (TEM).** TEM showing rod-shaped *H. pylori* (A), well-distributed cytoplasm and moderate electron density (B), U-shaped (C, arrow) and V-shaped (D, arrow) *H. pylori*, non-integrity (E, arrow) and abscission (F, arrow) of *H. pylori* cell wall, outer membrane separated from the cytoplasmic membrane (G, arrow), decreased electron density in cytoplasm (H, arrow), and cell lysis (I) after treatment with celecoxib.

**Table 2 mRNA levels in *H. pylori* flagellin, urease and adhesin genes meausred by real-time quantitative PCR (mean ± SD)**

| Gene | Celecoxib (0.02 mmol/L) | DMSO control (1/1000) |
|---|---|---|
| *flaA* | 23.08 ± 1.70[a] | 51.08 ± 6.91 |
| *flaB* | 16.01 ± 0.04[a] | 34.80 ± 7.13 |
| *ureA* | 19.61 ± 1.78[a] | 7.65 ± 0.38 |
| *ureB* | 29.59 ± 5.31[a] | 13.80 ± 1.63 |
| *babA* | 16.78 ± 0.91[a] | 12.38 ± 0.38 |
| *sabA* | 49.00 ± 4.10[a] | 22.55 ± 2.26 |
| *alpA* | 15.55 ± 0.78[a] | 7.34 ± 0.20 |
| *alpB* | 14.07 ± 0.23[a] | 8.95 ± 0.38 |
| *hpaA* | 123.98 ± 11.82[a] | 57.15 ± 2.56 |
| *hopZ* | 100.25 ± 4.37[a] | 45.54 ± 11.64 |

[a]$P < 0.05$ *vs* DMSO control.

mRNA expression levels in the above genes increased or decreased 1.5-2.5 folds in the presence of celecoxib (Table 2).

## DISCUSSION

NSAID and *H. pylori* infection are the two main etiological factors for peptic ulcers. However, their role in the pathogenesis of gastric mucosal damage is still controversial[6]. It has been demonstrated that eradication of *H. pylori* can decrease the recurrence rate of peptic ulcer and its complications in chronic NSAID users[14], while their co-existence aggravating gastric mucosal damage has not been confirmed[4,5]. It was reported that the prostaglandin synthesis level in mucosa is significantly higher in *H. pylori* positive patients than in *H. pylori* negative patients[15,16], demonstrating that colonization of *H. pylori* reduces the inhibitory effect of NSAID

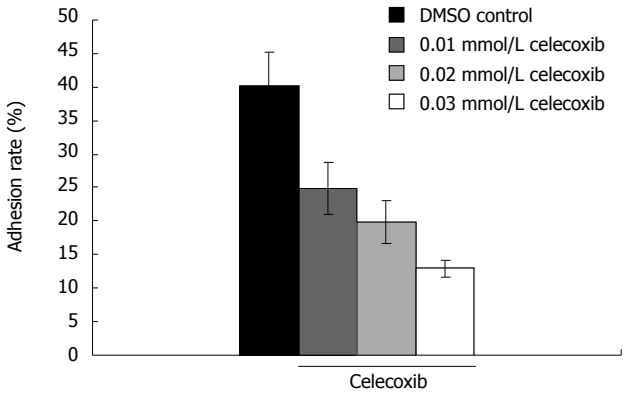


**Figure 3 Adhesion of *H. pylori* to AGS cells after treatment with celecoxib at different concentrations.**

on prostaglandin synthesis. *In vitro* studies further revealed that NSAID can inhibit the growth of *H. pylori*, and decrease the activity of urease and vacuolating cytotoxin[8-12], suggesting that NSAID may alter the pathogenicity of *H. pylori* in gastric mucosa injury when the two factors are co-existed in gastric mucosa.

*H. pylori* infection may persist for many years in the host and *H. pylori* colonization-related factors include its spiral shape, flagellar motility, urease and adhesin. Urease neutralizes the pH around *H. pylori* during exposure to the acidic lumen of stomach. The flagella and the spiral shape of *H. pylori* enable *H. pylori* strains to move and penetrate the mucin layer where they come into contact with gastric epithelial cells. Adherence of *H. pylori* to AGS cells is a crucial initial step in colonization[17,18], as non-adhering *H. pylori* strains would be washed away during peristalsis-mediated flushing of stomach. NSAID and celecoxib do not increase the colonization of *H. pylori* in gastric mucosa[19-23]. On the contrary, the incidence of *H. pylori* infection in patients taking NSAID is low[24,25], which may be partially explained by the fact that celecoxib can destruct the normal structure of *H. pylori,* and inhibit the motility of flagella, and the adherence of *H. pylori* to AGS cells and the activity of urease[12], which is consistent with the findings in our study.

Adhesin, exposed on the surface of *H. pylori* cells, facilitates interaction with host cellular receptors. The particularly more important adhesins of *H. pylori* are BabA, SabA, AlpA, AlpB, HpaA, HopZ[26-30]. Their content and expression under different environmental conditions are variable. In our *in vitro* study, celecoxib inhibited the adherence of *H. pylori* to AGS cells, but increased the mRNA expression levels in *babA, sabA, alpA, alpB, hpaA, hopZ*. Whether the increased mRNA expression in such genes is accompanied with an increased competent protein or just a compensatory increase in mRNA expression for the inhibition of *H. pylori* growth and adherence activity remains to be further studied. On the other hand, variable expression of cell receptors in a single host and genetic variability of receptor expression in different hosts make the adherence system very complex. Host receptor expression is up-regulated following *H. pylori* adherence[31]. In this study, the impaired adherence of *H. pylori* to AGS cells in the presence of celecoxib down-regulated the host receptor expression. In this condition, although the expression of *H. pylori* adhesins increases, the adherence of *H. pylori* to AGS cells may decrease.

Urease in *H. pylori* accounts for approximately 10% of the total bacterial protein pool[32]. Urease hydrolyzes urea and releases ammonia, which neutralizes acid, thus enabling survival and initial colonization. It has been shown that urease activity is essential for the initial bacterial colonization[33-35]. Anti-ulcer drug, ecabet, interferes with *H. pylori* colonization by inhibiting urease activity[36]. In the present study, celecoxib inhibited the urease activity in a dose-dependent manner, suggesting that it may further influence *H. pylori* colonization.

Urease is composed of two structural subunits, UreA and UreB. Urease gene clusters include *ure A, B, C, D, E, F, G, H, I,* with *ureA* and *ureB* being the structural genes. *ureC* and *ureD* are located before the structural genes. *ureI, ureE, ureF, urgG* and *ureH* are auxiliary genes. These genes and the structural genes are necessary for urease activity[37]. Urease is a metal enzyme possessing nickel and its activity depends on the two $Ni^{2+}$ inserted into its 6 active sites. The insertion process is accomplished by proteins encoded by auxiliary genes in a urease gene cluster. At present, a variety of identified proteins can regulate the activity of urease by influencing nickel ions. Besides proteins, different ion concentrations also accommodate urease activity[38]. Urease inhibitors can be generally classified into active site-directed (substrate-like) and mechanism-directed inhibitors. Since active site-directed inhibitors bridge the two paramagnetic nickel ions in the active site of urease, the octahedral nickel ions and the amino acid residues in the active site-directed inhibitors are in an orientation similar to those of the urease substrate, the mechanism-directed inhibitors are designed to interfere with the urease's catalysis mechanism leading to enzyme inactivation. In the present study, celecoxib inhibited the urease activity in *H. pylori*, but increased the mRNA expression levels in *ureA* and *ureB*. The mechanism still remains unclear. Further studies are needed to determine whether alterations occur at protein translation or modification level or some other mechanisms are involved.

The motility of *H. pylori* is considered another colonization factor. Less motile strains are less able to colonize or survive in the host than fully motile strains. It has been demonstrated that the degree of the motility of *H. pylori* strains is correlated with the degree of infectivity in gnotobiotic piglets. The most motile strains have a 100% infection rate, while the least motile strains have an infection rate of only 17%[39]. Strains without flagella or flagellar mutant strains cannot colonize the gastric mucosa, thus losing their pathogenicity. The flagella consist mainly of the flagellins, FlaA and FlaB.

Both genes coding for these flagellins are necessary for the full motility of *H. pylori*. Elimination of *flaB* yields normal-looking flagella that retain some functions and propel about 60% of the bacteria[40,41]. Elimination of *flaA* yields truncated flagella that only slightly move the bacteria. Elimination of both flagellins results in aflagellated immobile bacteria[41]. It was reported that NSAID inhibit the movement of *Proteus vulgaris, Proteus mirablis, Providencia rettgeri, Providencia stuartiii and Burkholderia cepacia* in a dose- dependent manner[42], and prevent emergence of *Escherichia coli* flagella by inhibiting flagellin synthesis[43]. In this study, celecoxib inhibited the motility of *H. pylori* and decreased the mRNA expression in *flaA* and *flaB*.

The relation between the degree of *H. pylori* motility, cytokine response levels and the severity of disease has been extensively studied[44,45]. The *H. pylori* motility levels are correlated with IL-8 induction[44]. Kurihara[45] also found that the degree of *H. pylori* motility is low in strains isolated from remnant gastritis, which is distinct from chronic gastritis, peptic ulceration or gastric cancer, indicating that the type and phase of *H. pylori*-related diseases dictate the selective pressure for maintenance of high *H. pylori* motility levels. Further study is needed to demonstrate whether celecoxib prevents the progress of *H. pylori*-related diseases by inhibiting *H. pylori* motility.

Besides the flagella, the shape of *H. pylori* strains makes them possible to penetrate the mucin layer where they come into contact with the gastric epithelial cells. In the present study, transmission electron microscopy showed that celecoxib could impair the formation of *H. pylori*, break the bacterial outer membrane, and destruct its structure. Since the spiral shape of *H. pylori* is one of the important virulence factors, celecoxib-related morphological changes may have an impact on the progress of *H. pylori*-induced diseases.

Gastric carcinoma is the forth most common cancer and the second leading cause of cancer-related deaths worldwide. The high mortality is largely attributed to the huge number of at-risk individuals. Chemoprevention appears to be the most promising approach in reducing the incidence and mortality of *H. pylori*-related gastric cancer. WHO defined *H. pylori* as a risk factor for gastric carcinoma and classified *H. pylori* strains as group I carcinogen in 1994[46]. The prevalence of *H. pylori* infection increases with age[47], and 50% of NSAID users are over 60-year old. NSAID contribute to the chemoprevention of gastric cancer and prevention of lymphatic metastasis by inhibiting angiogenesis and inducing apoptosis of epithelial cells through the COX-dependent and independent pathway. It has been shown that long-term intake of NSAID and aspirin can significantly reduce the incidence of non-cardial gastric cancer in a dose-dependent manner[48]. The results of our study further suggest that celecoxib can reduce *H. pylori* colonization, thus attenuating the pathogenesis in gastric mucosa. Although regular use of aspirin can prevent gastric cancer, it may be disadvantageous for populations with a lower risk of gastric cancer. Those with a high risk of gastric cancer can use celecoxib, a selective COX-2 inhibitor with few gastrointestinal side-effects.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Use of non-steroidal anti-inflammatory drugs (NSAID) and *Helicobacter pylori* (*H. pylori*) infection are the two main etiological factors for gastric injuries. Subjects taking NSAID are often co-infected with *H. pylori*, but the interaction between NSAID taking and infection with *H. pylori* remains unclear. Data from clinical and epidemiological studies are still controversial.

### Research frontiers

The relation between NSAID and *H. pylori* in the pathogenesis of gastric mucosal damage is still controversial. A number of studies have shown that it is not simply additive, synergistic or antagonistic. There may be complex interactions between them which affect the pathogenicity of each other.

### Innovations and breakthroughs

NSAID, as a harmful factor for gastric mucosal barrier, may be expected to increase the colonization of *H. pylori* in gastric mucosa. However, evidence from epidemiological studies indicates a lower prevalence of *H. pylori* infection in patients taking NSAID, which may partially be explained by the fact that celecoxib destructs the normal structure of *H. pylori*, and inhibits the flagellar motility, the adherence of *H. pylori* to AGS cells and the urease activity, as observed in this study.

### Applications

Colonization of *H. pylori* is a crucial initial step in the pathogenesis of *H. pylori* in gastric mucosa. The present study suggested that celecoxib could reduce the colonization of *H. pylori*, thus attenuating the pathogenicity in gastric mucosa.

### Terminology

SYBR green teal-time polymerase chain reaction (PCR): a quantitative PCR method for determination of the cope number of PCR templates such as DNA or cDNA in a PCR reaction. SYBR green: A dye that binds to the minor groove of double stranded DNA. When SYBR green dye binds to double stranded DNA, the intensity of fluorescent emissions increases. As more double stranded amplicons are produced, SYBR green dye signals increase.

### Peer review

The study described the effect of celecoxib on *H. pylori*. The study is well-designed. The experimental data are sufficient to support its conclusion.

## REFERENCES

1 **Telford JL**, Covacci A, Rappuoli R, Chiara P. Immunobiology of Helicobacter pylori infection. *Curr Opin Immunol* 1997; **9**: 498-503
2 **Walker MM**, Crabtree JE. Helicobacter pylori infection and the pathogenesis of duodenal ulceration. *Ann N Y Acad Sci* 1998; **859**: 96-111
3 **Antonov KI**, Isacson DG. Prescription and nonprescription analgesic use in Sweden. *Ann Pharmacother* 1998; **32**: 485-494
4 **Loeb DS**, Talley NJ, Ahlquist DA, Carpenter HA, Zinsmeister AR. Long-term nonsteroidal anti-inflammatory drug use and gastroduodenal injury: the role of Helicobacter pylori. *Gastroenterology* 1992; **102**: 1899-1905
5 **Stack WA**, Atherton JC, Hawkey GM, Logan RF, Hawkey CJ. Interactions between Helicobacter pylori and other risk factors for peptic ulcer bleeding. *Aliment Pharmacol Ther* 2002; **16**: 497-506

6 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22
7 **Hawkey CJ**, Tulassay Z, Szczepanski L, van Rensburg CJ, Filipowicz-Sosnowska A, Lanas A, Wason CM, Peacock RA, Gillon KR. Randomised controlled trial of Helicobacter pylori eradication in patients on non-steroidal anti-inflammatory drugs: HELP NSAIDs study. Helicobacter Eradication for Lesion Prevention. *Lancet* 1998; **352**: 1016-1021
8 **Gu Q**, Xia HH, Wang WH, Wang JD, Wong WM, Chan AO, Yuen MF, Lam SK, Cheung HK, Liu XG, Wong BC. Effect of cyclo-oxygenase inhibitors on Helicobacter pylori susceptibility to metronidazole and clarithromycin. *Aliment Pharmacol Ther* 2004; **20**: 675-681
9 **Wang WH**, Wong WM, Dailidiene D, Berg DE, Gu Q, Lai KC, Lam SK, Wong BC. Aspirin inhibits the growth of Helicobacter pylori and enhances its susceptibility to antimicrobial agents. *Gut* 2003; **52**: 490-495
10 **Wang WH**, Hu FL, Wong BCY, Berg DE, Lam SK. Inhibitory effects of aspirin and indometacin on the growth of Helicobacter pylori in vitro. *Chin J Dig Dis* 2002; **3**: 172-177
11 **Shirin H**, Moss SF, Kancherla S, Kancherla K, Holt PR, Weinstein IB, Sordillo EM. Non-steroidal anti-inflammatory drugs have bacteriostatic and bactericidal activity against Helicobacter pylori. *J Gastroenterol Hepatol* 2006; **21**: 1388-1393
12 **Ma HX**, Wang WH, Hu FL, Li J. Effect of aspirin and celecoxib on Helicobacter pylori in vitro. *Shijie Huaren Xiaohua Zazhi* 2006; **14**: 2747-2752
13 **Andersen LP**. Colonization and infection by Helicobacter pylori in humans. *Helicobacter* 2007; **12** Suppl 2: 12-15
14 **Chan FK**, To KF, Wu JC, Yung MY, Leung WK, Kwok T, Hui Y, Chan HL, Chan CS, Hui E, Woo J, Sung JJ. Eradication of Helicobacter pylori and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. *Lancet* 2002; **359**: 9-13
15 **Bhang CS**, Lee HS, Kim SS, Song HJ, Sung YJ, Kim JI, Chung IS, Sun HS, Park DH, Lee YS. Effects of selective cyclooxygenase-2 inhibitor and non-selective NSAIDs on Helicobacter pylori-induced gastritis in Mongolian gerbils. *Helicobacter* 2002; **7**: 14-21
16 **Hudson N**, Balsitis M, Filipowicz F, Hawkey CJ. Effect of Helicobacter pylori colonisation on gastric mucosal eicosanoid synthesis in patients taking non-steroidal anti-inflammatory drugs. *Gut* 1993; **34**: 748-751
17 **Mobley HL**. Helicobacter pylori factors associated with disease development. *Gastroenterology* 1997; **113**: S21-S28
18 **Chow J**, Ma L, Ch C. The role of adhesion molecules in gastric ulcer healing. *World J Gastroenterol* 1998; **4**: 467-468
19 **Caselli M**, Pazzi P, LaCorte R, Aleotti A, Trevisani L, Stab ellini G. Campylobacter-like organisms, nonsteroidal anti-inflammatory drugs and gastric lesions in patients with rheumatoid arthritis. *Digestion* 1989; **44**: 101-104
20 **Shallcross TM**, Rathbone BJ, Wyatt JI, Heatley RV. Helicobacter pylori associated chronic gastritis and peptic ulceration in patients taking non-steroidal anti-inflammatory drugs. *Aliment Pharmacol Ther* 1990; **4**: 515-522
21 **Graham DY**, Lidsky MD, Cox AM, Evans DJ Jr, Evans DG, Alpert L, Klein PD, Sessoms SL, Michaletz PA, Saeed ZA. Long-term nonsteroidal antiinflammatory drug use and Helicobacter pylori infection. *Gastroenterology* 1991; **100**: 1653-1657
22 **Loeb DS**, Talley NJ, Ahlquist DA, Carpenter HA, Zinsmeister AR. Long-term nonsteroidal anti-inflammatory drug use and gastroduodenal injury: the role of Helicobacter pylori. *Gastroenterology* 1992; **102**: 1899-1905
23 **Santucci L**, Fiorucci S, Patoia L, Di Matteo FM, Brunori PM, Morelli A. Severe gastric mucosal damage induced by NSAIDs in healthy subjects is associated with Helicobacter pylori infection and high levels of serum pepsinogens. *Dig Dis Sci* 1995; **40**: 2074-2080
24 **Taha AS**, Nakshabendi I, Lee FD, Sturrock RD, Russell RI. Chemical gastritis and Helicobacter pylori related gastritis in patients receiving non-steroidal anti-inflammatory drugs: comparison and correlation with peptic ulceration. *J Clin Pathol* 1992; **45**: 135-139
25 **Laine L**, Marin-Sorensen M, Weinstein WM. Nonsteroidal antiinflammatory drug-associated gastric ulcers do not require Helicobacter pylori for their development. *Am J Gastroenterol* 1992; **87**: 1398-1402
26 **Borén T**, Falk P, Roth KA, Larson G, Normark S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895
27 **Mahdavi J**, Sondén B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larsson T, Teneberg S, Karlsson KA, Altraja S, Wadström T, Kersulyte D, Berg DE, Dubois A, Petersson C, Magnusson KE, Norberg T, Lindh F, Lundskog BB, Arnqvist A, Hammarström L, Borén T. Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation. *Science* 2002; **297**: 573-578
28 **Odenbreit S**, Till M, Hofreuter D, Faller G, Haas R. Genetic and functional characterization of the alpAB gene locus essential for the adhesion of Helicobacter pylori to human gastric tissue. *Mol Microbiol* 1999; **31**: 1537-1548
29 **Evans DG**, Karjalainen TK, Evans DJ Jr, Graham DY, Lee CH. Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of Helicobacter pylori. *J Bacteriol* 1993; **175**: 674-683
30 **Evans DG**, Evans DJ Jr, Lampert HC, Graham DY. Restriction fragment length polymorphism in the adhesin gene hpaA of Helicobacter pylori. *Am J Gastroenterol* 1995; **90**: 1282-1288
31 **Bode G**, Malfertheiner P, Ditschuneit H. Pathogenetic implications of ultrastructural findings in Campylobacter pylori related gastroduodenal disease. *Scand J Gastroenterol Suppl* 1988; **142**: 25-39
32 **Bauerfeind P**, Garner R, Dunn BE, Mobley HL. Synthesis and activity of Helicobacter pylori urease and catalase at low pH. *Gut* 1997; **40**: 25-30
33 **Eaton KA**, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. *Infect Immun* 1991; **59**: 2470-2475
34 **Skouloubris S**, Thiberge JM, Labigne A, De Reuse H. The Helicobacter pylori UreI protein is not involved in urease activity but is essential for bacterial survival in vivo. *Infect Immun* 1998; **66**: 4517-4521
35 **Eaton KA**, Gilbert JV, Joyce EA, Wanken AE, Thevenot T, Baker P, Plaut A, Wright A. In vivo complementation of ureB restores the ability of Helicobacter pylori to colonize. *Infect Immun* 2002; **70**: 771-778
36 **Ito Y**, Shibata K, Hongo A, Kinoshita M. Ecabet sodium, a locally acting antiulcer drug, inhibits urease activity of Helicobacter pylori. *Eur J Pharmacol* 1998; **345**: 193-198
37 **Mobley HL**, Island MD, Hausinger RP. Molecular biology of microbial ureases. *Microbiol Rev* 1995; **59**: 451-480
38 **Pérez-Pérez GI**, Gower CB, Blaser MJ. Effects of cations on Helicobacter pylori urease activity, release, and stability. *Infect Immun* 1994; **62**: 299-302
39 **O'Toole PW**, Lane MC, Porwollik S. Helicobacter pylori motility. *Microbes Infect* 2000; **2**: 1207-1214
40 **Josenhans C**, Labigne A, Suerbaum S. Comparative ultrastructural and functional studies of Helicobacter pylori and Helicobacter mustelae flagellin mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in Helicobacter species. *J Bacteriol* 1995; **177**: 3010-3020
41 **Suerbaum S**, Josenhans C, Labigne A. Cloning and genetic characterization of the Helicobacter pylori and Helicobacter mustelae flaB flagellin genes and construction of H. pylori

flaA- and flaB-negative mutants by electroporation-mediated allelic exchange. *J Bacteriol* 1993; **175**: 3278-3288
42 **Kunin CM**, Hua TH, Bakaletz LO. Effect of salicylate on expression of flagella by Escherichia coli and Proteus, Providencia, and Pseudomonas spp. *Infect Immun* 1995; **63**: 1796-1799
43 **Farber BF**, Wolff AG. The use of nonsteroidal antiinflammatory drugs to prevent adherence of Staphylococcus epidermidis to medical polymers. *J Infect Dis* 1992; **166**: 861-865
44 **Watanabe S**, Takagi A, Tada U, Kabir AM, Koga Y, Kamiya S, Osaki T, Miwa T. Cytotoxicity and motility of Helicobacter pylori. *J Clin Gastroenterol* 1997; **25** Suppl 1: S169-S171
45 **Kurihara N**, Kamiya S, Yamaguchi H, Osaki T, Shinohara H, Kitahora T, Ishida H, Ozawa A, Otani Y, Kubota T, Kumai K, Kitajima M. Characteristics of Helicobacter pylori strains isolated from patients with different gastric diseases. *J Gastroenterol* 1998; **33** Suppl 10: 10-13
46 **Logan RP**. Helicobacter pylori and gastric cancer. *Lancet* 1994; **344**: 1078-1079
47 **Dooley CP**, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, Blaser MJ. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989; **321**: 1562-1566
48 **Wang WH**, Huang JQ, Zheng GF, Lam SK, Karlberg J, Wong BC. Non-steroidal anti-inflammatory drug use and the risk of gastric cancer: a systematic review and meta-analysis. *J Natl Cancer Inst* 2003; **95**: 1784-1791

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